ZINC-65 UPTAKE IN A TWO-STEP MARINE FOOD CHAIN

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A laboratory food chain study was conducted to measure the values of the parameters used to calculate the Maximum Permissible Concentration of zinc-65 in sea water. The food chain consisted of two microscopic marine algae, Nitzschia closterium and Carteria sp., and the bottom feeding fish, mullet (genus Mugil). The parameters evaluated were the rates of biological elimination of zinc by mullet, and the maximum concentrations of zinc-65 accumulated by the algae and mullet as a result of chronic exposure to this nuclide.

The algae concentration factors (uc per gm algae/uc per ml culture medium) were slightly greater for Carteria sp. than for Nitzschia closterium, the respective average values being 15,900 and 13,200. However, results indicated that the magnitude of these factors was limited by available zinc-65 rather than species characteristics.

Mullet uptake of zinc-65 from the algae was describable by a mathematical model, and the apparent maximum concentrations were reached in 55 to 60 days. These maximums decreased in magnitude as mullet size increased. For comparable size fish, the maximum mullet concentration factors relative to the algae culture medium were 230 for Carteria-fed fish compared to 135 for those fed Nitzschia cells. The magnitude of these factors indicates discrimination rather than concentration of zinc-65 by the mullet compared to the algae zinc-65 concentration. Differences in maximum uptakes of zinc-65 from the two algae were significant, but these differences were attributed to variation in zinc-65 and total zinc concentrations in the fish food rather than to differences in the mode of uptake from the two species of algae. Biological half-lives, which define elimination rates and affect maximum uptake values, varied from 33 days to 99 days depending on the method of measurement.

Mullet were found to have value as indicators of zinc-65 contamination of marine environments, and efficient monitoring programs could be designed on the basis of this property.

The Maximum Permissible Concentration of zinc-65 in sea water based on the results of this study would be approximately five times greater than the current recommended value of 7×10^{-9} uc/ml. However, similar evaluations of additional marine food chains are necessary before recommendations are made to raise the existing value.

CHAPTER I

DESCRIPTION OF THE PROBLEM

Rationale for Development of Maximum Permissible Concentrations of Zinc-65 in Sea Water

This investigation is a study of the transport of the radionuclide, zinc-65, through a two-step marine food chain leading to man. This food chain consists of two microscopic marine algae, Nitzschia closterium and Carteria sp., and mullet, a common marine food fish. The reason for the study is based on the fact that the radiations emitted by zinc-65 (primarily positrons and gamma rays) are potentially harmful to biological systems. These radiations can also be useful, as in the case of various applications for the study of physical and biological phenomena involving zinc. However, when zinc-65 is discharged to the environment as a waste product it becomes hazardous to man and lower organisms if its concentration in the environment exceeds safe limits. The hazard is two-fold. That is, if zinc-65 is present in the environment surrounding man, its radiations present an external hazard, and at the same time it can be ingested or inhaled and become an internal hazard.

Consequently, regulation of the amount of zinc-65 released to the environment is necessary.

The purpose of this study is to determine the values of parameters utilized in deciding how much zinc-65 can be discharged to the marine environment, a quantity referred to as the Maximum Permissible Concentration (MPC). MPC theory implies acceptability of risk. Otherwise, no decision would be necessary, because the MPC would simply be zero. The use of radiation to destroy cancer cells provides an example of such risk acceptance. Healthy tissue may be harmed in the treatment, but this risk is normally preferable to the potential damage from cancer. Establishment of an MPC for a radioactive waste such as zinc-65, however, is not so clear cut. To make this decision, the threat to human safety imposed by the radiation must be considered, keeping in mind the benefits to be gained from use of zinc-65 and the hardship on disposal organizations imposed by highly restrictive MPC's. Thomas (1964) illustrated this decision making process with a mathematical model. The most difficult parameter of his model to estimate was the utility of the human being in terms of economics. Decisions on the establishment of an MPC ultimately involve allowable health risks for given economic gains.

Standards have been proposed regarding acceptable amounts of radiation to the human body. The International Commission on Radiological Protection (ICRP) (1960) has recommended the amounts of about 240 radionuclides which can be tolerated in the body without serious damage. These maximum permissible body burdens serve as the basis for determining MPC values in the environment. The ICRP also has suggested MPC values for air and drinking water, based on a standard man whose water intake is 2200 ml per day and whose air intake is 2×10^7 cm^3 per day. If this standard man consumed water and breathed air contaminated at or below the MPC level for 50 years, the amount accumulated in his body at the end of the 50 years would not exceed the maximum permissible body burden.

The ICRP recommendations serve as basic tools which are used in establishing MPC's for the marine environment. Because sea water is not usually consumed directly by man, the contamination of sea food normally is the criterion which determines the level of a radionuclide that can be discharged initially to the water. Thus, transfer of the radionuclide from the water through one or more food organisms is a key determinant of MPC establishment. Such a series of organisms is referred to as a food chain in the following discussions. The transfer does not have to be unidirectional; recycling can be involved, thereby increasing the

complexity of the food chain mechanism. In fact, Schaeffer (1961) concluded that a major difference between terrestrial and marine food chains is the length and complexity of the latter.

Calculation of Maximum Permissible Concentrations

The National Academy of Sciences-National Research Council (NAS-NRC) (1960) summarized the steps involved in deriving MPC's for sea water based on the maximum permissible body burdens recommended by the ICRP. These steps or calculations take two forms dependent on where the damage to the body occurs. Internal hazard to humans from a radionuclide will result from radiation of the gastrointestinal (GI) tract as the nuclide passes through the body and from radiation of other body organs which accumulate the nuclide. For some radionuclides, the dose to the GI tract limits the maximum permissible body burden, whereas for others the accumulation in other organs is the limiting factor. The limiting organ is termed the critical organ.

As a radionuclide in food passes through the GI tract, no appreciable accumulation occurs in the tissue of the tract itself so that the radiation dose to the GI tract is a function of the concentration of the radionuclide in the food. On the other hand, if the nuclide is

accumulated by some organ, the radiation dose to that organ and surrounding tissue is dependent on the total amount of nuclide accumulated in the organ. This accumulation is governed by the specific activity (ratio of radioactive to nonradioactive atoms) of the nuclide in the food rather than the concentration. This is so because an organism does not normally distinguish between radioactive and nonradioactive isotopes of an element. Organisms in the sea will accumulate radioactive and nonradioactive nuclides in the same proportion as they exist in the water. Therefore, the specific activity of a radionuclide in a human body organ cannot exceed the specific activity of that nuclide in the sea. Rather, the specific activity in the body will be less than in the sea because of decay of the radioactive isotope during its passage through the marine food chain.

To identify the parameters governing the determination of MPC's in sea water, it is necessary to examine both methods of calculation; that is, the case for the GI tract as the critical organ and the case for some other organ being critical. The NAS-NRC (1960) has presented these calculations, and they are summarized here.

Case I. Critical body organ not the GI tract.

Definitions:

I_r -- radioactive isotope of element

I_n -- nonradioactive isotope of element

I_{rb} -- MPC of I_r in human body

I_{re} -- MPC of I_r in sea

I_{rf} -- concentration of I_r in sea food

I_{nb}, I_{ne}, I_{nf} -- concentrations of I_n in critical organ of human body, sea water, and sea food

K -- radioactive decay constant of I_r

B -- biological elimination constant for I_r and I_n in human body

B_f -- biological elimination constant for I_r and I_n in marine organisms

(Each of these decay or elimination constants is equal to $\frac{0.693}{T_{1/2}}$ where $T_{1/2}$ is the respective radioactive or biological half-life.)

F -- factor of concentration of I_r and I_n in marine organisms compared with sea water

C_r -- rate of intake of I_r by critical body organ

C_n -- rate of intake of I_n by critical body organ

If a = the fraction of ingested isotope taken up by the critical body organ and M = the mass of sea food eaten per unit time, then

$$C_r = aI_{rf}M$$

$$C_n = aI_{nf}M$$

The rate of uptake of the isotopes by the critical body organ can then be represented by

$$\frac{dI_{rb}}{dt} = C_r - (K + B)I_{rb}$$

$$\frac{dI_{nb}}{dt} = C_n - BI_{nb}$$

Uptake here is used in the sense of accumulation, which is the difference between the rate of intake by the organ minus the rate of loss.

The solutions of these equations, assuming no initial quantities of I_r and I_n are present in the organ, are

$$I_{rb} = \frac{C_r}{K + B}\left(1 - e^{-(K+B)t}\right)$$

$$I_{nb} = \frac{C_n}{B}\left(1 - e^{-Bt}\right)$$

It can be seen from these equations that as t becomes long compared to the half-life, $(K + B) = \frac{0.693}{T_{1/2\ \text{Effective}}}$ and $B = \frac{0.693}{T_{1/2\ \text{Biological}}}$, I_{rb} and I_{nb} will

approach equilibrium values given by $I_{rb} = \frac{C_r}{K + B}$ and $I_{nb} = \frac{C_n}{B}$. Therefore,

$$\frac{I_{rb}}{I_{nb}} = \frac{C_r}{C_n} \left(\frac{B}{K + B}\right)$$

Substituting for C_r and C_n

$$\frac{I_{rb}}{I_{nb}} = \frac{I_{rf}}{I_{nf}} \left(\frac{B}{K + B}\right) \qquad (1)$$

By similar reasoning, the equation for a marine organism accumulating an element from sea water becomes

$$\frac{I_{rf}}{I_{nf}} = \frac{I_{re}}{I_{ne}} \left(\frac{B_f}{K + B_f}\right) \qquad (2)$$

This argument can be extended to the case where one marine organism f_1 obtains an element from sea water (equation 2 above) and a second marine organism f_2 obtains the element from f_1 and finally the human body obtains the element from f_2. Thus,

$$\frac{I_{rf_2}}{I_{nf_2}} = \frac{I_{rf_1}}{I_{nf_1}} \left(\frac{B_{f_2}}{K + B_{f_2}}\right)$$

$$= \frac{I_{re}}{I_{ne}} \left(\frac{B_{f_1}}{K + B_{f_1}}\right)\left(\frac{B_{f_2}}{K + B_{f_2}}\right) \qquad (3)$$

Substituting equation 3 in 1,

$$\frac{I_{rb}}{I_{nb}} = \frac{I_{re}}{I_{ne}} \left(\frac{B_{f_1}}{K + B_{f_1}}\right) \left(\frac{B_{f_2}}{K + B_{f_2}}\right) \left(\frac{B}{K + B}\right)$$

$$= \frac{I_{re}}{I_{ne}} \left(\frac{BB_{f_1}B_{f_2}}{K^3+K^2(B+B_{f_1}+B_{f_2}) + K(BB_{f_1}+BB_{f_2}+B_{f_1}B_{f_2})+BB_{f_1}B_{f_2}}\right)$$

$$I_{re} = \frac{I_{rb}}{I_{nb}} I_{ne} \left(1 + \frac{K^3 + K^2(B+B_{f_1}+B_{f_2}) + K(BB_{f_1}+BB_{f_2}+B_{f_1}B_{f_2})}{BB_{f_1}B_{f_2}}\right) \quad (4)$$

This equation shows which parameters are essential for determination of I_{re}, the MPC for the radionuclide in sea water. The values of I_{rb} and I_{nb} are published by the ICRP (1960). I_{ne}, the concentration of the element in sea water, is usually known, as are K and B. Therefore, the biological elimination constants, B_{f_1} and B_{f_2}, for marine organisms are the only unknowns required for solution of the equation. Unfortunately, data on B_f are limited.

If the values for B_{f_1} and B_{f_2} are unknown, it is necessary to make conservative assumptions in order to simplify equation 4. For a radionuclide such as zinc-65, which has a relatively long physical half-life (245 days) (U. S. Department of Health, Education, and Welfare, 1960) and thus a small value of K, the most conservative assumption is that the B_f values are much larger than B and K. This amounts to saying that the hold-up time

in the marine organisms is short. Under this assumption, equation 4 reduces to

$$I_{re} = \frac{I_{rb}}{I_{nb}} I_{ne} \qquad (5)$$

Conservative values of I_{re} can therefore be calculated from known information. In so doing, it is possible to determine MPC's, which if followed, will assure that maximum permissible human body burdens are not exceeded. The problem is that such conservatism may impose unjust economic penalties on individuals or agencies concerned with disposal of radioactive materials. If the MPC selected is to be the most equitable balance between safety and utility, the biological elimination constants for marine organisms must be known. Determination of these constants for mullet is a primary objective of this food chain study.

Further considerations involved in determination of an MPC value for zinc-65 in sea water are brought out by the second case. That is, the situation in which the radiation dose to the GI tract limits the maximum permissible body burden and thus the MPC for a given radionuclide. Using the same terminology as for Case I, the development of an equation for this second case is summarized.

Case II. Critical body organ is GI tract.

Equation 3 can be solved for I_{re},

$$I_{re} = I_{rf_2} \frac{I_{ne}}{I_{nf_2}} \left(\frac{K^2 + K(B_{f_1} + B_{f_2}) + B_{f_1}B_{f_2}}{B_{f_1}B_{f_2}} \right)$$

but the concentration factor $F = \frac{I_{nf_2}}{I_{ne}}$

$$\therefore \quad I_{re} = \frac{I_{rf_2}}{F} \left[1 + \frac{K^2 + K(B_{f_1} + B_{f_2})}{B_{f_1}B_{f_2}} \right] \qquad (6)$$

I_{rf_2} is the concentration of the radionuclide in the sea food and is thus comparable to the MPC values for drinking water with the GI tract as the organ of reference. These MPC's are published by the ICRP (1960) and are based on a standard man whose water consumption is approximately 5 pounds per day. For the human being who obtains all his protein from sea food, the NAS-NRC (1960) estimates his consumption of sea food would be approximately 1/2 pound per day or 1/10 the water consumption. The MPC for sea food could therefore be increased by a factor of 10 over that for water. Substituting $10\ (MPC)_w$ for I_{rf_2}, equation 6 becomes

$$I_{re} \simeq \frac{10(MPC)_w}{F} \left[1 + \frac{K^2 + K(B_{f_1} + B_{f_2})}{B_{f_1}B_{f_2}} \right] \qquad (7)$$

In the event B_{f_1} and B_{f_2} are unknown, equation 7 can conservatively be reduced as was done under Case I to

$$I_{re} \simeq \frac{10(MPC)_w}{F} \qquad (8)$$

However, a new unknown, F, remains. Fortunately, more data are available on F values for marine organisms than B_f values, but much information in this area is still needed. Especially in the case of food chains there is considerable uncertainty regarding the effect a nuclide's passage through several organisms may have on the final concentration of the nuclide in a food organism. A second objective of this research is therefore to determine the concentration factor, F, for zinc-65 in mullet. The research is also designed to determine whether this concentration factor is a function of the organism consumed by fish and whether fish size influences the factor.

The NAS-NRC (1960) concluded that the most valid approach to calculation of a given MPC was to use both equations 5 and 8 and to select the lower value. Utilizing this technique, they recommended an MPC for zinc-65 in sea water of 7×10^{-9} uc/ml,[1] which was the value satisfying equation 5. This value is conservative, and it will be compared with the value determined by the exact equations 4 and 7.

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[1]The system of representing microcuries by the symbol, uc, is used throughout the dissertation rather than the newer designation picocuries (pCi).

Relation of Maximum Permissible Concentrations to Zinc-65 Detection Methods

If an MPC is to be practical, suitable methods must exist for determining the concentration of the radionuclide at the level of the MPC and preferably below this level. The recommended zinc-65 MPC of 7×10^{-9} uc/ml would be equivalent to approximately 0.02 disintegrations per minute (dpm) per ml. Reliable detection of this activity level even in large samples requires sensitive equipment and long counting or determination times. However, if zinc-65 is concentrated sufficiently and consistently by some organism, use of this organism as an indicator of low levels of zinc-65 in the sea water may be advantageous. A third objective of this research is to evaluate the indicator potential of the marine organisms comprising the food chain.

Relation of Maximum Permissible Concentrations to Tissue Distribution of Zinc-65

The distribution of the radionuclide in edible marine organisms is important. Consideration of any organism in establishing MPC's is predicated on the assumption that the nuclide concentrates to some extent in the edible portion of the organism. However, other tissues than flesh may also be important, because the nuclide may reach man indirectly from these tissues.

Examples would be the use of portions of fish as fertilizers and as sources of fish oil. Also, a consideration which may be of future importance is the possibility of using entire fish as a protein supplement. In this eventuality, activity in some inedible portion of the fish would be of equal concern with the flesh activity. These facts prompted the fourth objective of the research, which is to determine the distribution of zinc-65 among the various mullet tissues.

Summary

The purpose of this research is to evaluate current MPC values for zinc-65 in sea water. Four specific objectives are:

(1) To determine the biological elimination constant for zinc-65 in mullet.

(2) To determine the maximum concentration factor (radioactivity/unit dry weight of fish divided by radioactivity/unit dry weight of sea water) for zinc-65 in mullet. Two parameters which may affect this concentration factor are also evaluated; namely, the species of algae from which the mullet obtains the zinc-65 and the size of the mullet, which is related to age.

(3) To evaluate the potential of the algae and mullet as indicators of zinc-65 contamination of sea water.

(4) To determine the distribution of zinc-65 among mullet tissues.

CHAPTER II

A REVIEW OF THE LITERATURE

Zinc-65

Description and Physical Properties

Zinc-65 is the longest-lived radioactive isotope of this element. Its half-life is 245 days compared to a range of half-lives from 1.48 minutes for zinc-61 to 13.8 hours for zinc-69m (U. S. Department of Health, Education, and Welfare, 1960). (Unless otherwise stated all nuclear data for zinc-65 are taken from this reference.) This relatively slow decay rate results in zinc-65 being the only zinc isotope of appreciable importance from a waste disposal standpoint. The nucleus of this isotope contains 30 protons and 35 neutrons and decays by 1.5 per cent positron emission (0.324 Mev energy) and 98.5 per cent electron capture to copper-65. Some of the electron capture reactions are followed by a gamma ray of 1.12 Mev so that both positrons and gamma rays, as well as low energy X-rays (electron capture reactions), are produced in the decay process. Estimates of the total nuclear disintegrations attributed to gamma radiation vary from 44 to 48 per cent depending on the method of measurement. An average of 46 per cent was used

for the calculations in this research. Other physical and chemical properties of zinc-65 do not vary measurably from the properties of the stable zinc isotopes.

Production of Zinc-65

Artificially produced radioactive nuclides originate directly in one of two ways. They are either fission fragments produced when fissionable atoms are split, or they are formed by bombardment of atoms with nuclear particles (neutrons, protons, deuterons, and so forth). Zinc-65 is in this latter class of radionuclides. It is produced commercially in nuclear reactors for a variety of uses or as a byproduct through neutron activation of stable zinc in such substances as the cooling water of atomic reactors or the construction materials associated with nuclear bombs. An indirect method of formation involves production of gallium-65, which subsequently decays to zinc-65. Direct neutron activation of zinc-64 is, however, the most important source of zinc-65.

Although large quantities of radioactive zinc are produced for sale, only a small percentage of this total reaches the marine environment directly as waste because of U. S. Atomic Energy Commission (AEC) regulations limiting the uncontrolled release of such substances. Users of radioactive isotopes are required to return

their radioactive wastes to AEC approved facilities for disposal. Disposal of low levels of waste at sea after collection from isotope users is practiced, but these wastes are encased in concrete and the probability of their release to the ocean is slight.

However, a nuclear reactor or facility located near the ocean or on a river which empties into the ocean may release large amounts of radionuclides, including zinc-65, to the marine environment. Reactor cooling water is the primary source of such wastes. Much of the information on reactor waste releases is of a classified nature and not available to the public. However, numerous studies of this cooling water waste have been documented in the literature for the Hanford Works on the Columbia River in Washington. The NAS-NRC (1960) estimates from reported data that approximately 15 curies per day of zinc-65 empty into the river via the cooling water. A portion of this activity becomes associated with the river biota and sediments; the remainder reaches the Pacific Ocean. Some results of this discharge are reported in the next section.

In addition to land-based reactors, maritime reactors are potentially important sources of zinc-65 and other radioactive wastes. The NAS-NRC (1959) has published a report covering this problem based on an estimate of

300 nuclear-powered ships from all nations in service by 1975 with a potential total discharge of 100,000 curies per year of radioactive wastes. The zinc-65 content of these wastes was not estimated and would in any event be a minor fraction. However, when such large total quantities are involved, even minor fractions may be significant.

Other possible sources of zinc-65 contamination of the marine environment include *in situ* experimental studies involving tracer zinc and sewerage systems, which may receive low levels of zinc-65 waste. Nuclear accidents and planned nuclear detonations are not considered here as sources, because MPC values are not designed to cover these eventualities. Such possibilities cannot be ignored, however, for they can contribute large amounts of radioactivity to the oceans. It is conceivable that fallout, for example, in a small ocean area could raise the level of zinc-65 in the area above the established MPC value, thus reducing the usefulness of the MPC for its intended application.

The Importance of Zinc-65 in Biological Systems

Zinc-65's importance in biological systems must be considered from two standpoints: the effect of its radiations on the human body and its utilization by organisms which may transport it to man. The effect of radiations

on lower organisms than man is not normally a consideration, because these organisms can withstand much more radiation than a human being. For example, the Department of Health, Education, and Welfare (1962) reports that 600 roentgens or more of total body radiation will be lethal to 100 per cent of the human beings exposed to it, whereas 875 roentgens will kill only 50 per cent of exposed rabbits. Generally, as organisms become less complex physiologically, their radiosensitivity decreases.

Biological effects of radiation on the human body are dependent on the type of radiation, its energy, the rate and length of exposure, and the sensitivity of the tissues exposed. Zinc-65 has two principal radiations, a gamma ray of 1.12 Mev energy and a positron of 0.324 Mev energy. The damaging power of these radiations is a function of the energy which is transferred to exposed tissue. Such transfer decreases with increasing energy and inasmuch as energies in the Mev range are relatively high, the hazard from the gamma ray is low. The positron is a particle, however, and much more easily stopped by tissue than the gamma ray. For this reason, its relative hazard is high, but fortunately this hazard is partially offset by the low (1.5) percentage of the total disintegrations which are positrons. The resultant radiation hazard for zinc-65 is therefore low in comparison to other radionuclides. It is fortunate that this is so, because

zinc is readily taken up and concentrated by most body organs. The ICRP (1960) has indicated that three reasonable choices of a critical organ for zinc-65 are the total body, prostate gland, and liver, with respective maximum permissible body burdens being 60, 70, and 80 microcuries (uc). An idea of the relative radiotoxicity of zinc is obtained by comparing these values with the maximum permissible body burden of 2 for strontium-90.

Another zinc-65 characteristic of biological significance is the length of time it remains in body tissue. The factors K and B defined in Chapter I determine this residence time and are based on the physical and biological half-lives. The physical half-life of 245 days and biological half-life of 933 days result in an effective half-life of 194 days (ICRP, 1960). Thus, the radiation hazard from the nuclide may be low, but once ingested, it remains in the system for sufficient time to cause appreciable tissue damage.

Zinc-65 utilization by marine organisms is a function of the metabolic requirements of this metal and the total amount of zinc available to the organisms. The first factor establishes the organism's need for zinc and the second determines the degree of concentration in the organism relative to the sea. Each of

these factors promotes zinc concentration in marine organisms.

Considerable evidence regarding the biological functions of zinc indicates that it is an essential element. Taylor (1960), for example, studied the uptake of zinc-65 by marine phytoplankton. His results suggested that zinc was required for growth by Dunaliella euchlora. The studies of Provasoli and his co-workers (1957) on artificial media for culturing marine algae lead to a similar conclusion. They found that the addition of a number of trace elements, including zinc, to these media was necessary to achieve algal growth. Rice (1963) goes a step further by concluding that zinc is an essential nutrient for the growth of most microorganisms, plants, and animals.

The metabolic importance of zinc is related to enzyme systems which contain zinc as an integral part of the enzyme molecule or require catalysis by zinc and other divalent metals. The characteristics of seven zinc metalloenzymes and several other enzymes activated by zinc are discussed by Beerstecher (1964). Perhaps the most common of these enzymes is carbonic anhydrase, which catalyzes the reaction $CO_2 + H_2O \xrightarrow{\text{carbonic anhydrase}} H_2CO_3$ (Fruton and Simmonds, 1958). Therefore, this

enzyme and the zinc associated with it (0.33 per cent) are intimately involved in the basic life process of removal of CO_2 from cells. Several of the dehydrogenases also contain zinc and the transphosphorylases, phosphatases, arginase, and other enzymes require or are stimulated by Zn^{+2} and other divalent cations (ibid., 1958). Several modes of metabolic utilization of zinc in a given organism are consequently possible.

There is little doubt that most marine organisms will utilize available zinc-65 to some extent, but it is necessary to know how much they will use. Concentrations of stable zinc in sea water provide a partial answer. Assuming an organism has a relatively large requirement for an element, then the organism will tend to concentrate this substance if the sea water concentration is small. Zinc follows this pattern. Chipman, et al. (1958) compared zinc concentrations in the Atlantic Ocean at Beaufort, North Carolina, with concentrations at various other locations in the coastal areas in the United States. Values were all in the range of a few micrograms per liter (micrograms per liter is considered to be equivalent to parts per billion (ppb) in the following discussions), although some seasonable variations were evident at a given location. For example, concentrations at Beaufort ranged from 2.8 ppb in December to 14.6 ppb in August. Revelle and Schaeffer

(1958) report the average concentration of zinc in the sea as 10 ppb and the NAS-NRC (1960) in its MPC calculations used a value of 5 ppb. It would be expected then that the concentration of zinc in each level of a food chain would increase until an organism contained zinc in a concentration approximately equivalent to the consumer's requirement for the element on a unit weight basis. Parker's (1962) data support this contention. He studied the zinc distribution in a Texas bay. The water contained 8 to 15 ppb; algae and sediments concentrations ranged from 60 to 89 parts per million (ppm) and 10 to 18 ppm, respectively; and mullet, which feed on the algae and sediments, contained 130 ppm of zinc. For additional data on such concentration progressions, Rice's (1963) paper should be consulted.

Food Chain

Introduction

Except for some of the macroscopic algae, the sea food organisms utilized by man obtain at least a part of their food by ingesting other organisms. Any consideration of MPC's based on consumption of sea food should therefore involve the food chain which provides these organisms with nutrient materials. A food chain consisting of two microscopic marine algae, Carteria sp.

and Nitzschia closterium, and a bottom-feeding fish of the genus Mugil was chosen for this study. The elements of this food chain were selected primarily because of a desire to measure the greatest concentration of zinc-65 which could be achieved by a food chain. Microscopic algae are shown in a later section of this chapter to be among the highest concentrators of zinc-65 in the oceans and they constitute a primary source of food for mullet. A need for research on the uptake of radionuclides by detritus feeders such as mullet (Mugil) has been recognized by the NAS-NRC (1959). In their publication 658 they state:

> For food organisms which obtain all or part of their food by ingestion of detritus which has settled onto the bottom (for example the mullet, and some pelecypods and crustacea), we should take account of the concentration factors from bottom sediments to these organisms. Unfortunately, there are no data on this.

Algae

Carteria sp. and Nitzschia closterium are typical inshore species of marine algae and are cosmopolitan in their distribution. They represent respectively the green algae (Chlorophyceae) and diatoms (Bacillariophyceae) (Smith, 1950). Carteria sp., first described by Diesing in 1866, are solitary forms of the Volvocales and family Chlamydomonadaceae. Morphological features include four equal flagella, a green chromatophore, which is usually cup-shaped, and one or more pyrenoids. Possession of an eyespot is variable according to the species (ibid., 1950). Huber-Pestalozzi (1961) describes approximately 60 species of Carteria, whose cells range in length from 12 to about 30 microns. Cell protoplasm is encased in a cellulose wall, which makes rupture of the cell more likely than with an alga such as a diatom, a property which may affect the release of zinc-65 from the cells upon their death. It would be desirable to know the importance of Carteria as food for mullet and other fishes and the ecological relationships which would define its availability to these fish, but unfortunately almost nothing has been reported in the literature with regard to the ecology of marine species of this alga. Lackey and Glendenning (1965) found dense blooms of Chlamydomonas,

Platymonas, and other Volvocales in high tide pools of San Diego Bay, California, and concluded that the Volvocales were significant residents of this type of habitat. These pools, which are well fertilized by birds and decaying vegetation, provide ideal sanctuaries for mullet, especially in their juvenile stages.

Nitzschia closterium is a banana-shaped diatom with slender flexible ends and was first described by Ehrenberg in 1841 (Cupp, 1943). Each cell, varying in length from 25 to 100 microns, contains two yellow-brown chromatophores. In common with other pennate diatoms, N. closterium forms resting spores, which are tolerant of extremes in environmental conditions. Its cell walls are impregnated with silica and are very resistant to deterioration, even after death of the organism. Thus, zinc, which may be metabolically or physically bonded to the cells, may not be readily released to the environment upon death of the cell. As with Carteria, little work appears to have been done on the ecology of Nitzschia closterium, although this diatom has been utilized extensively in biochemical studies.

Representative cells of both species of algae are illustrated in Figure 1. Carteria cells are shown in a resting stage and have lost their flagella. When active,

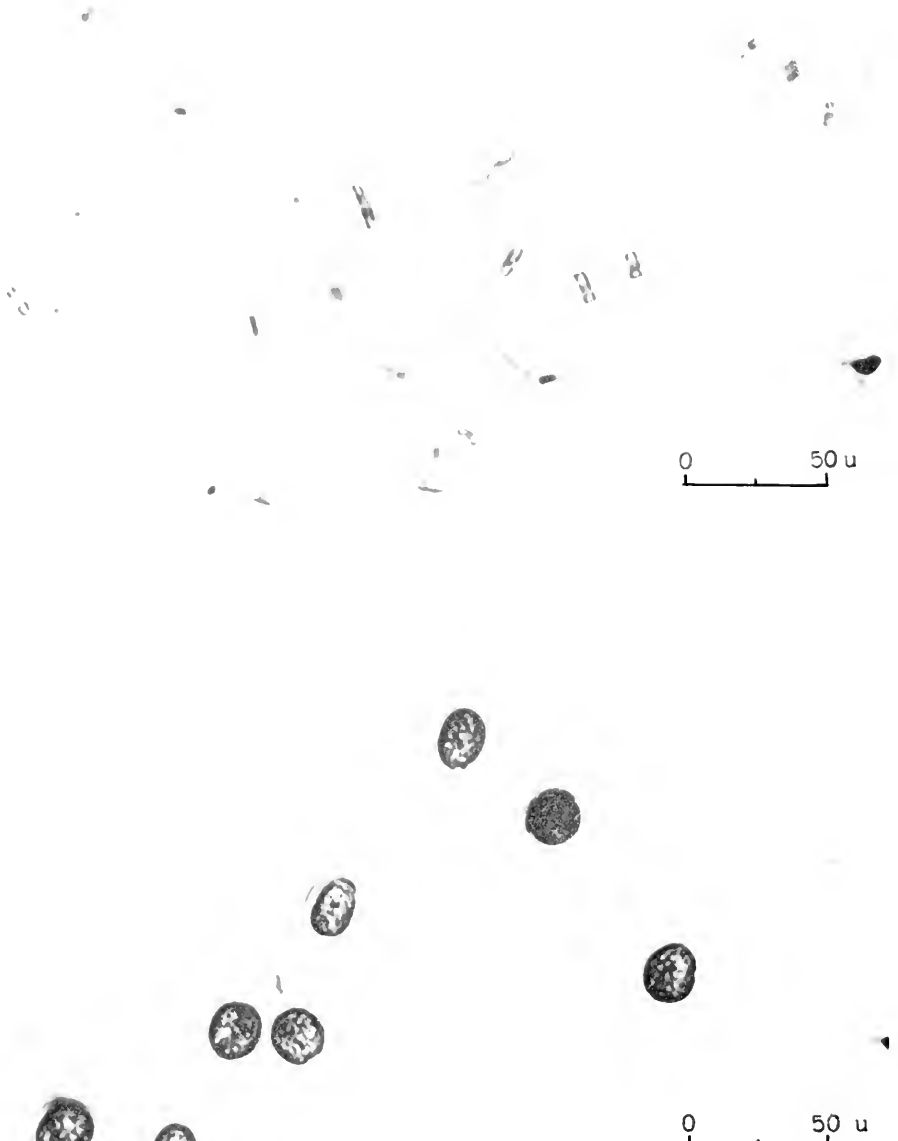

FIGURE 1
ALGAE CELLS
Nitzschia closterium (upper) - Carteria sp. (lower)

the cells do not possess the envelopes seen in the picture but other structural characteristics are the same.

Mullet

Mullet is a common name used to refer to two separate families of fishes, Mugillidae and Mullidae. The former are the subject of this research. Two species of Mugillidae, M. cephalus and M. curema, are the most common, being found in warm waters throughout the world (Encyclopedia Americana, 1957). Mullet, especially M. cephalus, abound in Florida waters and are an important food fish in this area. Broadhead (1953) estimated the value of the mullet catch at St. Marks West, Florida, at $450,000 per year. Some investigations pertaining to the ecology of mullet have been carried out, but almost nothing of the physiology of these fish is reported in the literature.

Migratory habits of this fish have particular bearing on their importance to the problem of zinc-65 contamination of the oceans. The mullet move from brackish pools and beach waters to the open oceans to spawn and the fry return to tidal pools and spend the majority of their juvenile stages there. Zinc-65, which might be carried continuously into these pools from a reactor waste

effluent, would be rapidly taken up by the algae and sediments and thus be available to essentially the same population of mullet on a continuous basis and at concentrations far above the sea water level. This type exposure would not be restricted to juvenile mullet, because even the adult mullet have a short migratory range. Idyll and Sutton (1951) found that the majority of tagged Mugil cephalus were caught within twenty miles of their spawning beds. It is apparent, then, that one necessary condition for the attainment of a maximum zinc uptake by mullet is met, namely, the opportunity of exposure for sufficient time to reach an equilibrium concentration. A situation providing evidence for this contention may exist in the lagoons of the Pacific atolls, which were involved in nuclear bomb tests in the early 1950's. Joyner (1962) found that zinc accounted for over 90 per cent of the activity in the tissues of mullet and other fish caught from one of these lagoons two to three years after cessation of the tests. Zinc-65 apparently persisted in this lagoon long after circulation and diffusion should have removed it. The mullet and other fauna of the area may have kept this isotope in active biological circulation as a result of uptake, excretion, and reuptake.

The feeding habits of Mugil also tend to reinforce their importance in zinc cycling in the environment. It has been pointed out already that they feed on algae but there appears to be little discretion in their diet. Although young fish seem to feed primarily on plankton, as they increase in age their diet shifts more to crustacea and higher forms (Taylor, 1951; Sarojine, 1954; and Reid, 1952). Adult mullet, however, have been observed feeding avidly on a bloom of the dinoflagellate, Gonyaulax sp., in the Gulf of Mexico (U. S. Department of the Interior, 1954) and from personal observation both young and adult mullet appear to take up detritus indiscriminately, expelling sand grains and other indigestible matter. Consequently, uptake of zinc-65 by the fish will not be dependent on a single or even a few food organisms.

These fish have a wide tolerance for environmental conditions. Kilby (1949), for example, collected mullet from water ranging in salinity from 1.1 parts per thousand (0/00) to 35.6 0/00 and has indicated in personal communication that they will tolerate salinities up to 80 per cent for short periods of time. The temperature range in the pools studied by Kilby was 13° to 44.5°C. and Zanibriborsch (1962) found an even lower temperature tolerance of 4° to 5°C. for a single species, M. auratus.

The occurrence of adverse conditions therefore may affect the uptake of zinc-65 by mullet, but probably will not be the limiting factor of the uptake.

Parameters of MPC Calculations

Biological Elimination Constants

Methods of Determination. It was seen in Chapter I that equitable balancing of safety versus utility in establishment of MPC values is dependent on knowledge of the rates of elimination of radionuclides from marine organisms. These rates are described by elimination constants, which are functions of the physical and biological half-lives for the radionuclide. Thus,

$$K = \frac{0.693}{T_{1/2\ \text{physical}}} \qquad \text{(physical decay)}$$

$$\text{and } B_f = \frac{0.693}{T_{1/2\ \text{biological}}} \qquad \text{(biological elimination)}$$

The two constants, K and B, are added to give the effective elimination constant so that

$$\frac{0.693}{T_{1/2\ \text{effective}}} = \frac{0.693}{T_{1/2\ \text{physical}}} + \frac{0.693}{T_{1/2\ \text{biological}}}$$

or

$$\frac{1}{T_{1/2\ E}} = \frac{1}{T_{1/2\ P}} + \frac{1}{T_{1/2\ B}}$$

This equation suggests an experimental method for determination of $T_{1/2}B$. An organism which has accumulated a quantity of a radionuclide may be placed in an environment free of the nuclide and its decrease in radioactivity monitored. The time required for the original radioactivity of the organism to decrease by one-half is a measure of $T_{1/2}$ E and inasmuch as $T_{1/2}$ P is a known constant, $T_{1/2}$ B and B_f can be calculated directly.

A second method is based on the equation from Chapter I describing the rate of uptake of a radionuclide by the human body. Applying this equation to marine organisms gives

$$I_{rf} = \frac{C_f}{(K + B_f)} (1 - e^{-(K+B_f)t}) \qquad (9)$$

and

$$I_{rf\ maximum} = \frac{C_f}{K + B_f} \qquad (10)$$

The equation can be rearranged to give

$$\frac{I_{rf}}{I_{rf\ max}} - 1 = -e^{-(K+B_f)t}$$

or

$$I_{rf\ max} - I_{rf} = I_{rf\ max}\ e^{-(K+B_f)t}$$

taking natural logarithms of both sides of the equation,

$$\ln (I_{rf\ max} - I_{rf}) = \ln I_{rf\ max} - (K + B_f)t \quad (11)$$

If an organism's uptake of a radionuclide can be monitored until it reaches a maximum value, the ln of $(I_{rf\ max} - I_{rf})$ can be plotted versus time. A resulting straight line will be evidence that the uptake follows equation 9 and the slope of this line will equal $-(K + B_f)$. Again, K is known, and so B_f can be calculated. A paper by Davis and Foster (1958) gives another method, which is a slight modification of this technique.

Existing Information. The preceding discussion points out that at least two methods exist for determination of zinc-65 (and other radionuclides) B_f values in marine organisms. Unfortunately, few controlled experiments have been reported in the literature to verify these models, a lack which has prompted this research.

Rice (1963) reports on unpublished data of T. J. Price, G. H. Rees, and D. E. Hoss regarding the respective retentions of zinc-65 by clams (Mercenaria mercenaria), blue crabs, and post-larval flounders (Paralichthys sp.). Effective half-lives, not tabulated, can be estimated from the graphs as approximately 30 days each for clams and crabs and 150 days for flounder. The clams obtained their zinc from sea water over a 20-day period; the crabs were given a single injection of isotope into the blood stream, and the flounder ingested the zinc-65 in food over a

91-day period. Because an organism which obtains a radionuclide over a long period of time would be expected to accumulate a large percentage of the nuclide in tissues with slow metabolic rates (muscle, bone, and so forth), elimination of the nuclide from chronically exposed organisms should be slow. The marked difference in retention between the crabs and clams and the flounder is probably a reflection of the differences in methods of nuclide administration as well as species differences. Rees also measured the difference between summer and winter retention by the crabs and found the winter excretion rate to be much slower (30 days versus 104 days), a result in accord with the decrease in metabolic rate of cold-blooded animals with decreasing temperature.

An experiment with results to which equation 11 can be applied is also reported by Rice (1963). Brine shrimp, Artemia salina, were allowed to accumulate zinc-65 from water and from food consisting of zinc-65 labeled algal cells in separate experiments. Recalculating the data to fit equation 11 and plotting the resultant numbers on a linear scale gives approximately parallel straight lines for both methods of uptake. The slope of the lines indicates an effective half-life of about six days for the shrimp. These results are

particularly interesting, because they indicate that excretion rates are not influenced by the source of the radionuclide.

Data on the decline of total radioactivity in fish and their tissues are presented by Weylander (1957). He collected samples of numerous species of fish, including mullet, from around Belle Island in the Eniwetok atoll from April, 1954, to November, 1955. This period encompassed the weapons testing program at the atoll in early 1954 and several months after the program's end. It is not possible to draw conclusions regarding elimination constants for specific radionuclides from his data, but he was able to conclude that the decline in radioactivity of all species was generally similar. However, it appeared that the decline of radioactivity in omnivorous fish was somewhat more rapid than in carnivores. Apparently, by the time radioactivity is transferred through the food chain to the carnivores, the short-lived radioactivity is gone and these fish ingest primarily long-lived nuclides. Their radioactivity decline would thus be expected to be slow.

A significant study by Mishima and Odum (1963) measured the effect of temperature and body size on the excretion rate of the salt marsh snail, Littorina

irrorata. Their experimental technique was particularly good in that care was taken to prevent reingestion of excreted zinc and the data were analyzed by careful statistical techniques. Results showed that excretion varied directly with temperature and inversely with body size. No significant interaction between temperature and body size was indicated. Furthermore, it was observed that the excretion rate, initially rapid due to probable loss of nonassimilated zinc, slowed as the tracer was eliminated from tissue. A third and even slower rate step was becoming evident at the termination of the experiment after 39 days. The zinc-65 was administered to the snails in a single dose; had it been fed over a longer period of time, it is probable that the third rate would have been more predominant. Biological half-lives for the largest snails were estimated to be 40, 25, and 23 days at the respective temperatures of 15, 25, and 30°C.

In addition to temperature, the effects of varying amounts of food intake and of salinity on excretion rates of small marine fish were measured by Shulman, Brisbin, and Knox (1961). Half-lives were 13 days, 58 days, and 44.5 days for Menidia menidia, Fundulus heteroclitus and Tautogolabrus adspersus at 20°C. and 35 0/00 salinity. Presumably these are effective

half-lives, although the authors do not clarify this detail. Excretion rates varied directly with temperature, whereas no effect by salinity or food intake could be demonstrated.

Odum (1961) found that half-lives for terrestrial insects decreased 5-fold when the insects were released from the laboratory to the field. He concluded this was due to greater insect activity in the field, disregarding the possibility that more stable zinc was available in the field resulting in faster replacement of the radioactive zinc.

The establishment of excretion rate constants for microscopic algae must be approached with caution. To use elimination constants in the context of this research presupposes that the biological half-life characterizing the constant is a fraction of the life span of the organism. With microscopic algae this is not a valid presumption. A given algal cell may divide several times in the space of a few days so that the maximum activity which cells may attain can be limited by their division rate. In addition, biological elimination constants predicated on some form of metabolic use of an element may be in error. Recent evidence in the literature shows that many microscopic and macroscopic algae take up zinc-65 by some nonmetabolic sorption mechanism

(Gutknecht, 1965, and Bachman, 1963). These considerations may explain in part the near absence of microscopic algae elimination data in the literature. Some information was reported by Chipman, et al. (1958). They suspended zinc-65 labeled Nitzschia closterium cells in nonradioactive medium and could detect very little loss of radioactivity from the cells. However, when the complexing agent, ethylenediamine tetraacetic acid (EDTA), was added to the medium the cells lost 89 per cent of their activity in 48 hours. This result would be reasonable if zinc-65 was physically rather than metabolically bound to the cells.

Macroscopic marine algae (seaweeds) do not pose the problems that microscopic forms do and some information is available on their excretion rates. Gutknecht (1965) reports zinc-65 half-lives ranging from four days for Ulva lactuca to 100 days for Fucus vesiculosus in a study involving nine different species of algae. It is not clear whether these are effective or biological half-lives. Growth rate was found not to affect excretion rates, although the paper does not present data in this area. It is interesting to note that the uptake by Ulva and Porphyra fitted the Freundlich adsorption isotherm suggesting nonmetabolic uptake of zinc and yet measurable half-lives

were observed for both algae. This latter characteristic implies metabolic turnover of the zinc or an exponentially time-dependent physical release of the element. It is probable that both metabolism and physical adsorption are involved.

Concentration Factors

Methods of Determination. Determination of zinc-65 concentration factors (F) for organisms which have accumulated the nuclide is straightforward. Measurement of the zinc-65 gamma radioactivity in the organism is relatively easy even on live specimens, and the ratio of this radioactivity on a unit weight basis to that of sea water is the factor, F. This ease of determination probably accounts for the reasonably large number of investigations which have been carried out to determine these factors. Most studies have been relatively short-term experiments involving acute rather than chronic exposures to zinc-65, resulting in little available information on maximum F values attainable as a result of chronic exposure via food chains. As will be seen in the following section, the data vary widely, partly due to biological differences and partly as a result of the nature of the

parameters which define F. These data are useful, however, because they point out the magnitude of the F values for various groups of organisms.

Existing information regarding concentration factors is significant when considered in relation to the criteria which determine the particular values of these factors. Referring to the equations in Chapter I, it may be seen that the maximum concentration of a nuclide in the body (I_{rb}) is a function of C_r, the rate of intake, and the physical and biological elimination constants, K and B. That is,

$$I_{rb} = \frac{C_r}{K + B}$$

Applying this equation to marine organisms,

$$I_{rf_2} = \frac{C_{rf_2}}{K + B_{f_2}} \qquad (12)$$

but $$C_{rf_2} = aI_{f_1}M$$

$$\therefore \quad I_{rf_2} = \frac{aI_{f_1}M}{K + B_{f_2}}$$

The maximum attainable concentration of a radionuclide in a marine organism which obtains the nuclide metabolically is therefore a direct function of at least

five factors: the mass of food ingested per unit time (M); the concentration of the nuclide in that food (I_{rf_1}); the fraction of the ingested nuclide which is retained (a); and the elimination constants (K and B_{f_2}). Wide variations in the magnitude of F values for different organisms are therefore to be expected. Obviously, investigation of all combinations possible with equation 12 for every marine food organism is not practical. However, the only factors in the equation intrinsic to the organism consumed by man are (a) and (B_f). Characterization of these parameters for the major sea food organisms is possible, and with this knowledge, limits could be placed on I_{rf_2} values by estimating M and I_{rf_1}. Unfortunately, no data are available in the literature on the values of (a) for marine organisms; researchers have been concerned primarily with F values.

Existing Information

Representative results of several investigators for different marine forms are summarized in Table 1.

It may be seen from the table that microscopic marine algae concentrate zinc-65 to a high degree, and Chipman, et al. (1958) report an extremely high value for oysters. Evidence is strong that uptake

TABLE 1

CONCENTRATION FACTORS FOR MARINE ORGANISMS

Organism	Concentration Factor	Wet or Dry Weight Basis	Type Exposure	Reference
Algae - Microscopic:				
Ochromonas sp.	7,600	Dry	Single Dose	Morgan (1961)
Platymonas sp.	53,800	Dry	Single Dose	Morgan (1961)
Novicula confervacea	23,600	Dry	Single Dose	Morgan (1961)
Chlamydomonas sp.	20,000	Dry	Single Dose	Morgan (1961)
Nitzschia sp.	42,000	Dry	Single Dose	Morgan (1961)
Rhodomonas sp.	312	Dry	Single Dose	Morgan (1961)
Nitzschia closterium	50,000	Wet	Single Dose	Chipman, et al. (1958)
Algae - Macroscopic:				
Fucus vesiculosus	3,300	Wet	Chronic Dose	Gutknecht (1965)
Codium decorticatum	30	Wet	Chronic Dose	Gutknecht (1965)
Mollusca:				
Oyster - Crassostrea virginica	25,000	Wet	Chronic Dose	Channell (1960)
Oyster - Crassostrea virginica	250,000	Wet	Single Dose	Rice (1963) from Chipman, et al. (1958)
Crustacea:				
Shrimp - Artemia salina				
Male	76	Wet	Chronic Dose	Rice (1963)
Female	125	Wet	Chronic Dose	Rice (1963)
Fish:				
Mullet - Mugil cephalus	16,250		Based on stable zinc analyses of fish and water	Parker (1962)

by microscopic and macroscopic algae may be largely due to nonmetabolic processes (Gutknecht, 1961, 1963, 1965; Chipman, et al., 1958; Bachman, 1963). As Gutknecht and Bachman point out, however, metabolic use of zinc by algae is not excluded as a possibility. Consequently, algal F factors may be influenced both by the parameters described at the beginning of this chapter (elimination constants, food intake and nuclide content, and retention factors) and by other criteria controlling nonmetabolic adsorption. Evaluation of these nonmetabolic controlling variables is not an objective of this dissertation, but it is an area deserving study.

Metabolic processes undoubtedly have a pronounced effect on zinc uptake in the higher organisms as evidenced by distinct concentration of the nuclide in internal organs (Chipman, et al., 1958). Additional support of this statement is suggested by the good fit of the metabolic equation $\left[(I_{rf} = I_{rf\ max}\ (1 - e^{-(K + B_f)t})\right]$ to Rice's (1963) uptake data for Artemia salina which was described in the preceding section. Consideration of the fraction (a) and elimination constant (B_f) for sea food organisms is therefore pertinent to a discussion of marine MPC's and sea food concentration factors.

Of the experiments indicated in Table 1, the data for Artemia salina are the most appropos to this dissertation. These shrimp were given a chronic exposure to zinc-65 via the marine alga Carteria sp. and a similar exposure via sea water. The Carteria cells had previously been grown in a medium containing the same concentration of zinc-65 as the sea water to which the shrimp were exposed. When the shrimp reached an equilibrium value, the algae-fed shrimp contained 2.6 times the amount of zinc-65 in the water-exposed individuals. Considering that the Carteria undoubtedly concentrated the zinc-65 considerably, this difference in F values is expected. The magnitude of I_{rf_1} (equation 12) was radically different for each case; so it would have been surprising had the F values been comparable. Hoss (1964) conducted a similar experiment using nauplii of Artemia salina as food for post-larval flounder and observed concentration factors 1.6 times as great in the nauplii-fed fish. The nauplii contained approximately 100 times more zinc-65 per gram than the water (estimated from Hoss's data) as opposed to a probable factor in the thousands for the Carteria used by Rice. Thus, even disregarding the different species involved, it

is not surprising that the difference between water and food uptake was somewhat smaller in Hoss's experiment than in Rice's.

Although a marine environment is not involved, practical field evidence of the facility with which zinc-65 is transferred and concentrated through aquatic food chains is provided by data on zinc-65 deposition in aquatic flora and fauna of the Columbia River below the Hanford Works (Davis, et al., 1958). The zinc-65 concentration of the water and a number of organisms collected from a station on the river is indicated in Table 2. The concentration factors, not reported in the paper, have been calculated for comparison with other data.

TABLE 2

ZINC-65 CONTENT OF COLUMBIA RIVER ORGANISMS

Organism	Zinc-65 Concentration uc/gm wet weight	Zinc-65 Concentration in Water uc/ml	Concentration Factor
Green Algae	1.23×10^{-2}	8.9×10^{-8}	1.38×10^{5}
Sponge	1.46×10^{-3}		1.64×10^{4}
Insect Larvae	1.98×10^{-3}		2.22×10^{4}
Snail	1.53×10^{-3}		1.72×10^{4}
Crayfish	3.65×10^{-4}		4.10×10^{3}
Minnows	7.62×10^{-4}		8.55×10^{3}

It cannot be said with certainty that these factors are maximum values inasmuch as the organisms' ages are not known, but the reactor had been in operation approximately ten years at the time of sampling, allowing ample exposure time for equilibrium to be reached. It is significant that the magnitudes of these concentration factors assessed under actual field conditions are comparable in most cases to the laboratory data of Table 1.

Tissue Distribution

The discussion pertaining to MPC parameters has so far been concerned with the quantitative aspects of the problem. This and the subsequent section deal qualitatively with two aspects.

The importance of determination of the distribution among tissues of the total zinc-65 accumulated by an organism has been pointed out in Chapter I. Previous studies provide considerable information on such distributions.

Some of the earliest work was concerned with marine life collected from the Pacific Ocean in the area of the nuclear bomb tests conducted in the 1950's. Numerous separate scientific expeditions to these areas

during and after the tests were made by scientists of this country and Japan. Cohn, Robertson, and Conrad (1960) found that zinc-65 contributed 90 per cent of the total radioactivity of the muscle and skeletons of fish collected from Rongelap Lagoon one to two years after the tests. By 1959 this activity in fish muscle had resulted in body burdens of 44 millimicrocuries (muc) of zinc-65 in two natives who had eaten fish from the lagoon. Evidence of this kind emphasizes that food chain transfer of zinc-65 to man does occur and is not just supposition.

Lowman (1963) reported similar results on fish collected in 1958 from the Eniwetok Proving Grounds. Percentages of total activity in muscle and liver attributed to zinc-65 in flying fish and tuna were:

	Liver	White Muscle	Dark Muscle
Tuna	78.5*	90.4*	87.1*
Flying Fish	9.9	58.8	

*Average of two species.

These data, compared to other radionuclides, show that zinc-65 accounts for the majority of activity found in the edible portions of sea food. However, they reveal little regarding the relation of muscle to other tissue activity.

Mori and Saiki (1956) studied the distribution of zinc-65 among various tissues by feeding the nuclide to carp (Cyprinus carpio) in a laboratory study. The fish were left in zinc-65 contaminated water for 22 days and then sacrificed and separated into 11 tissue components. The activity of each tissue expressed as counts per minute per gram from highest to lowest was kidney - 299, gill - 285, scales - 65, heart - 57, skin - 51, caudal fin - 50, intestine - 27, hepatopancreas - 26, vertebra - 5, and muscle - 3. A similar trend (low muscle content of zinc-65 and higher activities in kidney and liver) existed in a sucker (Catostomus machrocheilus) caught in the Columbia River (Davis, et al., 1958). These workers separated the retina of the eye from other tissues, however, and found that it contained three or more times the zinc-65 present in any other tissue. Joyner (1962) also found the retina to be the highest concentrator of zinc-65 in goatfish and convict surgeon fish collected in 1958 and 1959 at the Pacific proving grounds. It may be seen in the following section that this property, whereby zinc-65 concentrates in retinal tissue, may be important to the indicator potential of these fish.

Chipman, et al. (1958) investigated the distribution of zinc-65 in tissues of croakers as a function of time following administration of the nuclide. As would be expected, the muscle and bone gradually increased in activity whereas organs such as the liver rapidly reached a maximum and then decreased steadily. For example, muscle activity at zero time was 0.01 muc/gm and had increased to 2.21 muc/gm after 48 hours. On the other hand, liver activity at 6 hours was 57.46 muc/gm but at 48 hours had decreased to 11.72 muc/gm. Obviously, this shift in distribution is relevant to the health hazard of zinc-65 and reemphasizes the importance of long-term chronic exposure experiments.

Indicator Potential of Marine Organisms

Joyner (1962) proposes that the value of organisms as indicators of environmental contamination is dependent on four criteria: (1) the availability of a nuclide to the organism; (2) the physical half-life of the nuclide; (3) the biological half-life of the nuclide; and (4) the residence time of the nuclide in the environment. To this list should be added the demand of the organism for a nuclide, the variation of demand among individuals of the same species, the availability of the organisms to man, and the ease of

measurement of the radioactivity in the organism. Ideally, it would be desirable to collect an organism easily, determine its zinc-65 concentration with high precision in a short amount of time, and then be able to state with confidence that this concentration is a specific fraction of the zinc-65 concentration in the sea water. Realistically, it is not yet possible to attain this end with a biological system. The degree to which this goal is attained will determine the usefulness of any indicator organism.

Considering the danger of generalizing about radionuclide uptake by any organism, the paucity of literature on this subject is not surprising. Only two references were found dealing specifically with this subject.

Lackey and Bennett (1963) considered the indicator problem from the standpoint of whether certain microorganisms might show avoidance reactions to areas of high radioactivity so that the presence or absence of the organisms would indicate the corresponding absence or presence of radioactivity. No such correlation could be found due to the high resistance of microorganisms to radiation damage and their inability to distinguish between radioactive and nonradioactive isotopes of the heavier elements.

Although Joyner (1962) was not primarily concerned with indicator organisms, he found that the eyes of goatfish and surgeon fish from contaminated areas contained high concentrations of zinc-65. The fraction in the eyes was also relatively constant in accord with the proportionately constant metabolic activity of the eye. He concluded, therefore, that fish eyes had definite value as indicators of sea water contamination.

The attractiveness of the oceans as disposal areas for zinc-65 and other radionuclide disposal is almost certain to increase with time. Consequently, monitoring the level of contamination present will assume increasing importance, making the value of observations such as Joyner's more obvious. These should be tested on other organisms soon in order to validate this indicator potential and exploit it to the fullest possible extent.

Summary

Zinc-65 is an induced radionuclide, the decay of which produces a gamma ray of 1.14 Mev energy and a positron of 0.342 Mev energy. The relatively low hazard of its radiations is partially offset by its

ready uptake by body organs and long biological and radioactive half-lives.

Zinc is required by most organisms because of its association with enzyme systems. Low concentrations of zinc in sea water, coupled with strong zinc requirements, result in pronounced concentration of zinc-65 by marine organisms.

Inasmuch as an MPC must take account of the worst possible conditions from the standpoint of human safety, it would be desirable to study a food chain in which the uptake of zinc-65 is a maximum. The degree to which the food chain chosen for study meets this condition is not known, but the characteristics of algae and mullet would tend to promote rather than restrict uptake.

At least two experimental methods exist for determining zinc-65 biological elimination constants of marine organisms. Estimates of effective half-lives, which characterize these constants, range from less than 10 to 100 or more days, depending on the organism and numerous other conditions. Elimination rates vary directly with temperature and inversely with body size in cold-blooded animals, whereas salinity and food intake were not shown to have an effect. It is difficult to measure elimination rates from unicellular organisms and little information is available on this subject.

The maximum concentration of zinc-65 which an organism will attain by metabolic uptake of the nuclide is a function of at least five parameters. Two of these parameters, (a) and (B_f), are inherent properties of an organism; one, (K), is a constant; and the remaining two, (M) and (I_{f_1}) are related to the organism's environment. Existing information on concentration factors determined in the laboratory is comparable to field observations. However, much effort is needed to determine (a) and (B_f) values so that some generalization can be made for groups of organisms without inordinate amounts of experimental work. Research is also needed regarding effects of chronic exposure of organisms to zinc-65 via natural food chains so that realistic maximum F values can be obtained for marine food organisms.

The literature indicates that after a few weeks zinc-65 will be a predominant isotope in marine organisms exposed to a mixture of radionuclides. Fortunately, the concentration in edible portions of sea food is low, but this fraction increases steadily with time of exposure. The highest concentrating organ in fish appears to be the eyes, a property which is relevant to the value of fish as indicator organisms.

CHAPTER III

METHODS

Problem Approach

The intent of this research was to simulate by laboratory technique the subjection of a natural marine food chain of microscopic algae and mullet to continuous zinc-65 exposure for sufficient time to permit these organisms to achieve maximum uptake of zinc-65.

Specific objectives were achieved by culturing the algae, Nitzschia closterium and Carteria sp., in media containing zinc-65, incorporating the radioactive algae into fish food, and feeding them to young mullet in a controlled, statistically designed experiment; a separate experiment was conducted to measure zinc-65 elimination rates of the mullet. The research involved three phases: (1) the culturing and harvest of algae; (2) feeding of radioactive algae to mullet; and (3) monitoring of mullet zinc-65 elimination. Methods involved in each phase are discussed.

Algae

The algae, Nitzschia closterium and Carteria sp., were available from the culture museum at the Earle B. Phelps Laboratory, University of Florida. Although the cultures were not axenic, work by Morgan (1961) indicated that uptake of zinc-65 by bacteria was minor compared to uptake by microscopic algae. Use of nonaxenic cultures was therefore consistent with the research objectives.

The algae were cultured under batch conditions utilizing 5-gallon Pyrex carboys as the culture flasks. Inasmuch as zinc has been shown to adsorb readily on glass and other surfaces (Morgan, et al., 1964), the insides of these flasks were coated with silicone grease to reduce this adsorption to a minimum. Coating was conveniently achieved by dissolving a small amount of grease in carbon tetrachloride and swirling this solution around the walls of the flask. A uniform grease film remained after evaporation of the carbon tetrachloride and interference with light transmission was not significant when the film was thin. Experiments were carried out in an incubator room in which the temperature normally varied between 15 and 20°C. and in which a continuous light source was provided by a

bank of twenty 40-watt Sylvania fluorescent bulbs of the GRO-LUX variety. These light fixtures provided an illumination of 200 foot candles at the surface of the culture flasks as measured by a Weston Model 703, Type 6A light meter. Temperature control at 17 $\pm$ 3°C. was provided by the thermostat included with an air conditioning unit installed in the room.[1] The medium in the flasks was stirred constantly by magnetic stirrers at a speed just sufficient to keep the majority of the algae from settling or adhering to the sides of the flasks. Exact rotation speed was not measured but corresponded to approximately midrange on the H. G. Thomas No. 9235-C Magnetic Stirrer. Stirring motors were insulated from the flasks by sheets of heavy asbestos paper.

A medium (Appendix 1) suggested by Rice (1953) was used for algal culture. Aged sea water of 25 0/00 salinity was used in preparing the medium. The zinc concentration of this sea water was measured before and after addition of nutrients by a dithizone extraction technique used at the U. S. Fish and Wildlife Service Laboratory in Beaufort, North Carolina (Duke, 1965). A polarographic method of comparable accuracy to the

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[1]See discussion of the fifth algae experiment in Chapter IV for an exception to this generalization.

dithizone extraction method later became available (Morgan and Gubbins, 1965) and was used for all subsequent zinc analyses. Details of specific application of the polarographic method to sea water and other samples collected in this research are reported in Appendix 2 along with the dithizone technique.

For each algae dosing experiment the flasks were filled with 15 liters of sea water, nutrient solutions[1] A and B added, and the solutions autoclaved (121°C. for 15 minutes) and placed in the incubator room to cool to ambient temperature. Nutrient solution[1] C was then added and the cultures inoculated with 50 ml of a one- to two-week old subculture. Stirring was begun immediately and the cultures were allowed to grow for 12 hours before dosing with zinc-65. At 12 hours and each 24 hours thereafter, sufficient zinc-65 was added to the cultures to provide a concentration in the culture flask of 7×10^{-5} uc/ml which is 10^4 times the currently accepted maximum permissible concentration for zinc in sea water (NAS-NRC, 1960). Just prior to each dose of zinc-65 and one hour afterwards, a 5-ml sample of the culture was taken for determination of radioactivity and a 10-ml sample for cell count. Total radioactivity of the 5-ml sample was determined

[1]See Appendix 1 for the composition of these solutions.

and the algae activity measured separately after filtration onto a Millipore membrane filter (pore size 0.45 u). Cell counts were made by the drop sedimentation method of Lackey (Standard Methods, 1960). When the cell count reached a maximum, dosing of the culture was discontinued and the algae were harvested.

Following a quiescent period harvesting was accomplished by centrifugation at approximately 2000 rpm ($\sim$ 1000 x gravity) in an International floor model centrifuge. Carteria cells settled rapidly and quantitatively, but the majority of Nitzschia cells remained suspended. The cells plus medium were then siphoned from the bottom of the culture flask into 250 ml plastic centrifuge bottles. This technique required centrifuging of only a small fraction of the total medium to obtain the majority of the Carteria cells, but with Nitzschia, it was necessary to centrifuge the entire 15 liters. Each centrifugation took approximately 10 minutes, the entire 15 liters requiring from six to eight hours to harvest. The centrifuged algae were washed with nonradioactive sea water to remove any loosely adhering activity, stored in polyethylene bottles, and frozen. Just prior to freezing, triplicate samples of the harvested algae were taken for determination of radioactivity and moisture content. These determinations were made by placing the algae on tared copper

planchettes (one inch in diameter), weighing the planchettes, drying at 100°C. for several hours, obtaining the dried weight, and gamma counting the dry samples.

In order to determine the effects of centrifugation and freezing on the algae, aliquots of the frozen cells were resuspended in Rice's medium. Although some of the cells were ruptured in the harvesting process, the majority were intact and these resuspended cultures shortly began active growth and reproduction. This result is not surprising, as many forms of algae are known to remain viable at freezing temperatures (Lewin, 1962).

Mullet

Collection

Two species of mullet, *M. cephalus* and *M. curema*, were used and no attempt was made to separate the two. The mullet were caught in tidal marsh pools along the East and West Coasts of Florida. One pool near the Intercoastal Waterway at Crescent Beach, Florida supplied the majority of specimens. A cast net of 1/4-inch mesh was the usual means of obtaining the fish, although at times a 20-foot 1/4-inch mesh minnow net was found to be helpful in penning the mullet into a small area. Fish in the size range of 7 to 12 cm were kept, examples of which are pictured in Figure 2. Inasmuch as it is

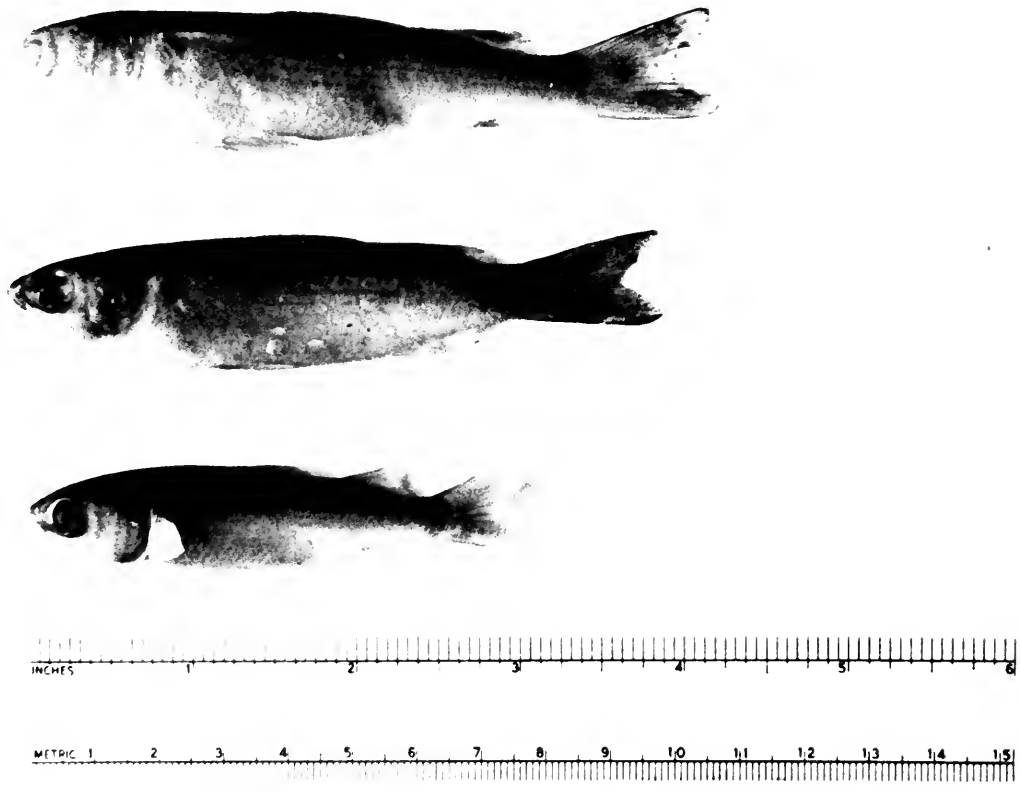

FIGURE 2
EXAMPLES OF MULLET

the mullet's habit to move to deeper water when the summer water temperatures decrease, it was necessary to obtain the mullet during the warmer months and hold them for later use. After collection, the mullet were placed in 5-gallon cream cans for transportation from the coast to the laboratory in Gainesville. Loss of fish during the trip was minimized by aerating the water in the cans occasionally with pure oxygen from a small supply cylinder. At the laboratory the fish were initially placed in a large outdoor aquarium and subsequently moved to individual laboratory aquaria.

Aquarium Systems

Two different aquarium systems were utilized in the study: an outside holding system consisting of a single aquarium with a capacity of up to 560 gallons, and a laboratory system in which the experiment was conducted. Both systems utilized recirculation without addition of makeup water, although distilled and tap water were added for salinity control. Those interested in additional information about salt water aquaria should consult Sea Water Systems for Experimental Aquariums (1964).

The outside aquarium system consisted of the aquarium, a hold-up reservoir, a sand filter, and the required piping and pump for recirculation and aeration. The system is shown schematically in Figure 3.

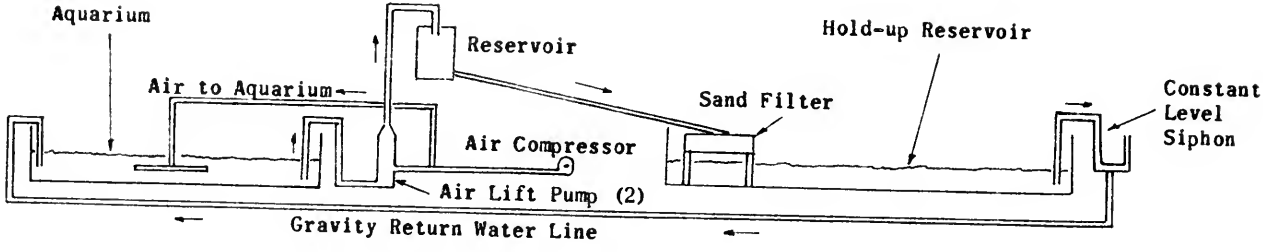

FIGURE 3

SCHEMATIC OF OUTSIDE AQUARIUM SYSTEM

The aquarium was a circular tank 8 feet in diameter by 2 feet deep fashioned from a plastic-lined wading pool obtained from the Sears Roebuck Company. It was installed on a 2-inch sand base in accordance with the manufacturer's instructions, and exposed metal parts covered with epoxy paint. To provide a somewhat natural environment for mullet, approximately 4 inches of mud obtained from the tide flats at Cedar Key, Florida, were placed on the bottom of the aquarium. A partial cover of black plastic over the aquarium restricted algal growths and provided a semi-dark environment which the mullet prefer, and further, the entire aquarium was sheltered by an open structure consisting of a wooden framework covered with plastic-coated window screen.

An existing, rectangular, cured concrete tank (13.5 feet long by 3 feet wide by 2 feet deep) was used as the hold-up reservoir. Water entered the tank through the sand filter at the end nearest the aquarium and was returned to the aquarium by gravity from the opposite end. This tank was also covered by black plastic.

The sand filter was constructed of wood and was placed on legs inside the hold-up tank. Filter details are represented schematically in Figure 4.

By using air lift type pumps, aeration and re-circulation was accomplished with one air compressor,

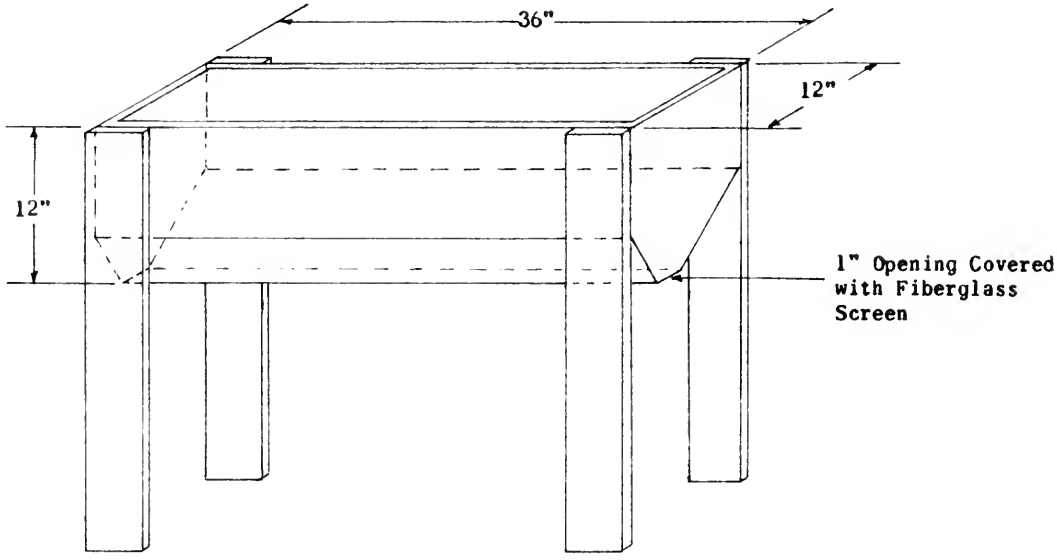

FIGURE 4

DETAILS OF OUTSIDE SAND FILTER

sold by the Fisher Scientific Company under their Serial No. 11906. The air lift pumps were constructed of glass tubing to raise the water 2 feet to a 1-gallon polyethylene reservoir. From this reservoir, the water flowed through rigid plastic pipe by gravity to the sand filter and into the hold-up reservoir at a rate of approximately 10 gallons per hour. The recirculation compressor pumped air to the aquarium through three diffuser stones attached to a glass manifold, and both the aeration and recirculation were controlled by an automatic timer on the compressor. The systems are represented in Figure 3.

The inside aquarium system in principle was identical with that outside, although the inside system was composed of 16 individual glass aquaria. Water was added separately to each aquarium and removed through siphons into discharge headers which emptied into a filter. Figure 5 is a schematic of the system.

The aquaria were rectangular glass tanks 11 inches long by 6 inches wide by 5.5 inches deep with a capacity of 6 liters. During the experiment they were kept approximately two-thirds full. Aquaria were arranged in four rows with four aquaria per row in a rectangular tank constructed of plywood and painted with epoxy paint. The purpose of this tank, which was equipped with a drain to the filter, was to contain the system in the event of

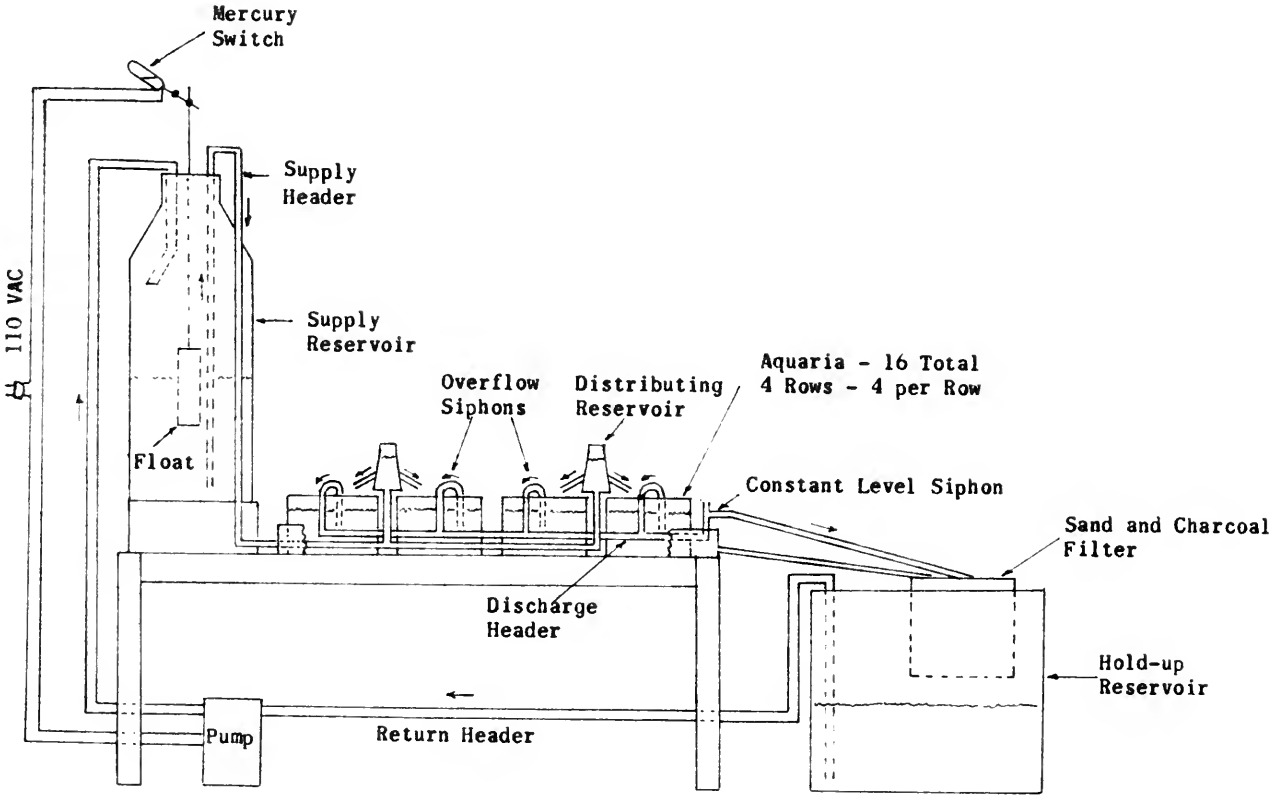

FIGURE 5

SCHEMATIC OF INSIDE AQUARIUM SYSTEM

breakage or malfunction. Each aquarium was fitted with a fiberglass screen cover to prevent the mullet from jumping out.

Two reservoir systems were used to distribute water to the aquaria. A large polyethylene carboy with a capacity of approximately 40 liters served as the main distributing reservoir and contained a float which actuated switches controlling the recirculation pump. From this central reservoir, water flowed by gravity to each of four small glass reservoirs constructed of 125 ml Erlenmeyer flasks. Through side arms, each of these small flasks supplied water separately to four aquaria and functioned as air traps so as to prevent air locks in the lines.

Three discharge headers received water from the aquaria through siphons, and emptied into a sand and charcoal filter through constant leveling devices. These devices, which were necessary to prevent all the water from siphoning out of the aquaria, have been used elsewhere (U. S. Department of the Interior, 1964).

A necessary requirement of the entire system was use of materials inert to sea water. For the filter, a 12-inch diameter section of vitrified clay pipe with a 2-inch collar was found to be ideal. The end opposite the collar was covered with fiberglass screen and the

pipe filled with approximately 12 inches of sand and 4 inches of activated charcoal. The filter was suspended over the hold-up tank by cutting a 12-inch hole in the plywood cover and inserting the pipe through the hole, the collar of the pipe thus acting as a retaining ring. During the experiment, the charcoal was replaced and the top two to three inches of sand washed once a week. However, the filter could be used without washing for up to a month without apparent effect on the water quality.

The hold-up tank was fashioned from a 55-gallon oil drum with the top one-third cut off. Two coats of epoxy paint provided satisfactory corrosion protection.

Water was cycled automatically from the hold-up tank to the main distributing reservoir by a pulsating pump, Sigmamotor Model No. T63. Air was continually supplied from the building supply to the hold-up tank through a manifold and diffuser stones. It was not necessary to aerate the aquaria.

With this system, 16 aquaria could be operated continuously without supervision other than for routine maintenance. In the feeding experiment only 12 aquaria were used, one row being held in reserve for the elimination experiment. The flow rate through the aquaria could be varied over wide ranges, but was normally about 100 ml per minute per aquarium.

Aquarium water temperature was not controlled but remained in the range of 23 to 25°C., and natural buffering kept the pH in the range of 7.55 to 7.75 during the course of the experiment. Prior to the experiment, it was thought that this pH might be too low and marble chips were added to the hold-up tank in an effort to increase the pH above 8, but no change was effected. The mullet became well acclimated to the lower pH and no further adjustments were attempted, although it was determined that the pH could be readily controlled by the addition of sodium carbonate.

Salinity was kept in the range of 35 to 40 0/00 by periodic additions of distilled water to the system. Although closer control of salinity could have been achieved, control of fungi was enhanced by allowing the salinity to increase periodically.

Food

The fish were maintained on an artificial food consisting of beef liver, dry baby cereal (Pablum), and canned spinach. The food was prepared by homogenizing one pound of beef liver and one 15-ounce can of spinach. This mixture was then kneaded into 8 ounces of the dry cereal, rolled into convenient packages, wrapped in aluminum foil, and boiled for five minutes to coagulate the blood in the liver. The cooked food was frozen and

the desired amounts shaved off as needed when feeding the fish. Soluble and fine particulate matter was eliminated by shaving the food into a small amount of water and decanting off the liquid. This was advantageous in reducing aquarium fouling. In order to feed the radioactive algae to the fish, the algae were substituted for a portion of the spinach in this mixture. The weight proportions chosen were one-fourth algae, one-fourth spinach, one-fourth liver, and one-fourth baby food. Heating the algae was avoided by boiling the spinach and liver homogenate and cooling the mixture to just above freezing before blending in the algae and baby food. The resultant mixture was packaged as before and immediately frozen. Triplicate samples of the frozen food were placed in copper planchettes, dried, and the moisture content and radioactivity determined. To evaluate the effect of the preparation procedure on the algae, samples of the fish food were suspended in sea water and the suspended algal cells examined under the microscope. Photographs of these resuspended cells are included in Figure 6. As can be seen, cells of both species remained intact.

In order to feed the same amount of food to fish each day, the prepared diet was divided into one gram packets. Each packet of fish food was wrapped separately in glassine weighing paper and kept frozen. Possible

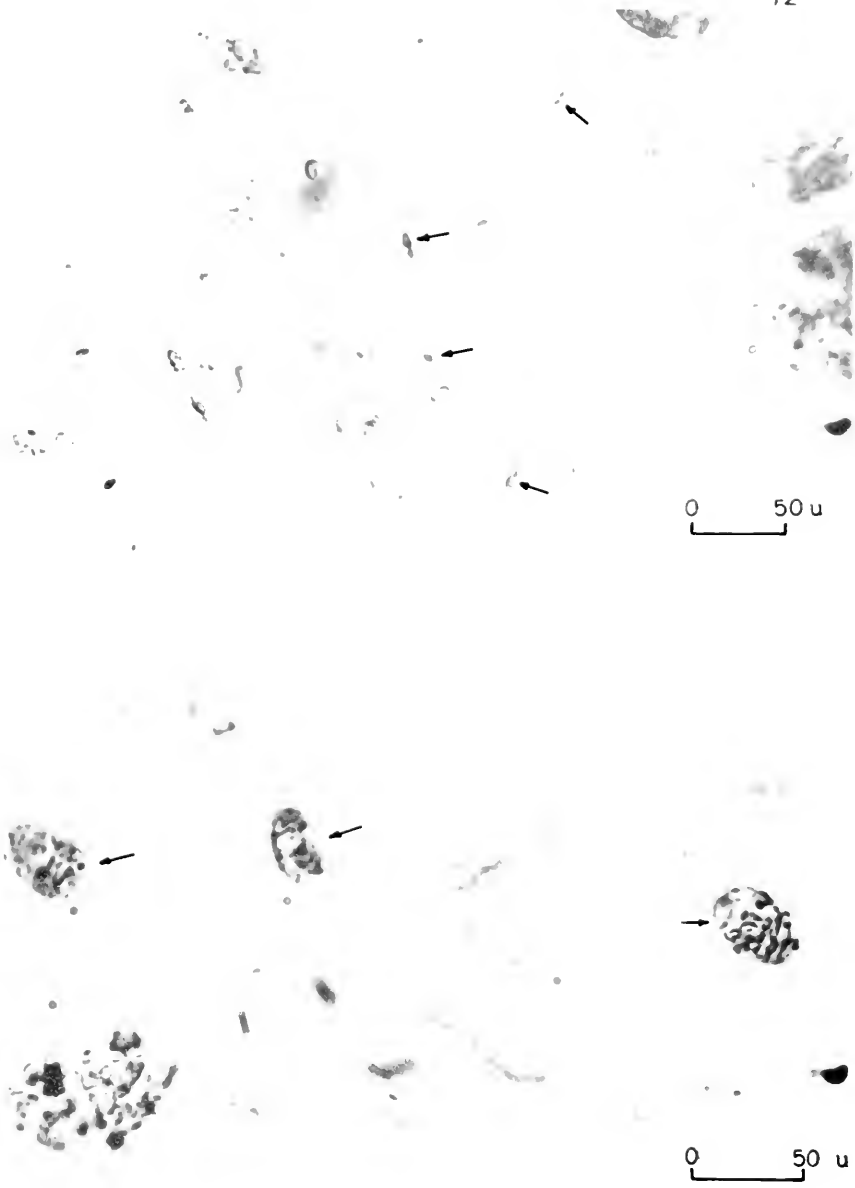

FIGURE 6
ALGAE IN FISH FOOD
Nitzschia closterium (upper) - Carteria sp. (lower)

spoilage of the fish food due to thawing and refreezing in this weighing process was avoided by keeping the food on a block of dry ice except when weighing. Weights were obtained to ± 0.1 of a gram. Prior experience had indicated that 0.5 gram of this fish food mixture was the approximate amount a fish would eat in a day without leaving an undue amount of residue in the aquarium. In the experiment a one gram package of food was added daily to each aquarium containing two fish.

Experimental Design

Statistics

The uptake phase of the experiment was conducted according to a completely randomized statistical design with fish size as a covariate (Snedecor, 1956). Fish size has been correlated with age in mullet by other investigators (Kilby, 1949; Broadhead, 1958). The fish were placed in the aquaria as they were taken from the outside holding aquarium. It was assumed that this process was essentially random in that no attempt was made to select particular fish. Randomization of treatments to aquaria was achieved by assigning random numbers from a standard random number table (Snedecor, 1956) to each aquarium. The numbers were then ranked and the first

four aquaria received Nitzschia fish food, the second four Carteria, and the third four, serving as controls, got nonradioactive food.

Experimental Techniques

Duplicate 5-minute activity determinations were made on each fish at periodic intervals. Temperature, pH, and salinity of the aquarium water were measured each time the fish were counted and at intervals in between. Samples of the aquarium water were taken once a week for stable zinc analyses and 5-milliliter samples of the influent and effluent of the filter were taken at each fish counting for determination of zinc-65.

The feeding schedule was continued and the accumulation of activity in each fish monitored until this activity reached a maximum in all fish. The fish were then sacrificed by immersion in MS-222 anestnetic (1000 ppm), frozen, and their zinc-65 content determined. At this time the fork length and the weight of each fish were recorded. Weights were taken after drying the fish thoroughly with paper toweling.

Each fish was dissected carefully into twelve parts: scales, skin, bone, muscle, gills, eyes, heart, liver, spleen, alimentary tract (gizzard, pyloric caeca, intestine), ingested food, and viscera (remainder of gut contents including kidney). The dry weight of each portion

was obtained and the dried tissue gamma counted in a scintillation well crystal.

In the elimination phase of the experiment eight fish, four per algae species (one Carteria-fed fish subsequently died), were fed radioactive algae for 33 days and then their diet was changed to nonradioactive food and their activity decrease followed for 21 days. Radioactivity determinations were made on the water at the beginning and during the experiment as a check on possible reingestion of excreted zinc-65.

Counting was achieved by placing the fish in a plastic cylinder 6 inches in length by 2 inches in diameter which was filled with nonradioactive sea water and to which air could be supplied during the course of the counting. Reproducible geometry was provided by placing the chamber in a polystyrene form which could be fitted over the 4" x 4" NaI scintillation crystal. The counting chamber is pictured in Figure 7. In order to facilitate handling during counting and to prevent injury to the fish, an anesthetic, trichane methane sulfonate, was added to the water at a concentration of 1:15,000 (67 ppm). This concentration did not produce total unconsciousness in the majority of the fish but generally resulted in immobility. Upon return to the aquarium water the fish were completely normal to all appearances

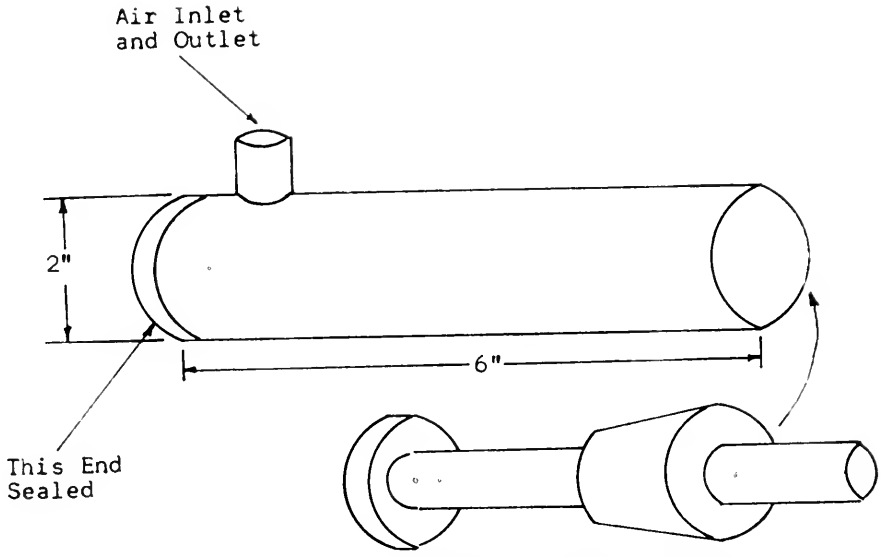

FIGURE 7

MULLET COUNTING CHAMBER

in from 30 seconds to one minute. Mr. Cliff Townsend, Curator of the Marine Research Laboratory at Marineland, Florida, indicated in a personal communication that he had observed no physiological effects on various marine fish even after repeated anesthetizing with this compound. The anesthetic is produced under the trade designation MS-222 by the Sandoz Pharmaceutical Company of Hanover, New Jersey. As a precaution against bacterial and fungal infections caused by repeated handling of the fish, 300 ppm of sodium sulfathiazole was also added to the aquarium water and the water used in counting the fish. Fish were first taken from the aquarium and placed in a solution of the anesthetic. After immobilization, taking from two to five minutes, they were washed in nonradioactive sea water and transferred to the counting chamber.

Radioactivity determinations in both algae and fish were carried out by gamma spectrometry techniques. The equipment used consisted of either a 2" x 2" sodium iodide well crystal or a 4" x 4" sodium iodide solid crystal coupled with a Nuclear of Chicago Model Number 1810 single channel analyzer and the scaling circuit of a Nuclear of Chicago Model Number 186 scaler. Counts were made with either a 10 per cent or 2.5 per cent

window covering the photopeak for zinc-65. The efficiency of the counting system was determined under various geometries by preparing known solutions of zinc-65 and counting them each time determinations were run. Table 3 indicates samples of the efficiencies obtained for the various geometries utilized. Inasmuch as some uncertainty existed in the absolute activity of the zinc-65 utilized in preparation of the standards, a check was run by performing an integral count on a cobalt-60 standard from the beginning of the first photopeak of cobalt-60 (1.17 Mev) to infinity. The efficiency determined in this manner was found to be identical with that determined using zinc. Undoubtedly these identical efficiencies were coincidence, but it was considered that this technique provided a sufficient check of the zinc standards.

TABLE 3

TYPICAL INSTRUMENTAL EFFICIENCIES FOR DETERMINATION OF ZINC-65

Type Source	Crystal	Window	Theoretical Activity	Measured Activity	Per Cent Efficiency
1" Copper Planchette	2"x2" - Top	10 %	70,500 ɣ/min.	976	1.4
5 ml Liquid in Plastic Test Tube	2"x2" - Well	10 %	14,100 ɣ/min.	532	3.8
5 ml Liquid in Glass Test Tube	2"x2" - Well	2.5%	8,250 ɣ/min.	371	4.5
Paraffin Fish in Counting Chamber	4"x4" - Top	10 %	2,820 ɣ/min.	26.0	0.9

CHAPTER IV

RESULTS AND DISCUSSION

Introduction

For ease and clarity of reading, the majority of tabular data is included in Appendices 3 and 4 and only those tables essential to the text remain in this chapter. Results are presented in the following order: (1) labeling algae cells with zinc-65, (2) feeding labeled algae to mullet, and (3) measurement of the elimination rate of zinc-65 from mullet. Mullet used in (2) and (3) are termed, respectively, uptake fish and decay fish, and results pertinent to each are discussed and tabulated by reference to these terms.

Algae

Results

Five experiments were conducted in which 1.05 uc of zinc-65 were added daily to rapidly growing 15-liter cultures of N. closterium and Carteria sp. Each

culture was allowed to grow until the number of cells per unit volume reached a maximum, at which time addition of zinc-65 was stopped and the culture harvested. The primary purpose of repeating experiments was to obtain sufficient algae for all mullet experiments and not for estimation of reproducibility. Table 4 summarizes the results for individual cultures.

The important data in Table 4 are the concentration factors which were determined by dividing the uc/gm of dry algae by the initial medium zinc-65 concentration (7×10^{-5} uc/ml). Factors for Nitzschia closterium (excluding the second experiment) varied from 10,700 to 16,150 compared to a range of 13,000 to 17,700 for Carteria sp. Corresponding averages are 13,200 and 15,900. The only variable intentionally changed in these experiments was the specific activity of the zinc-65 sources. A source of 15.9 ugm/uc soecific activity was used for the first experiment and a 1.7 ugm/uc source for the remaining four. The zinc concentration of the culture medium (79 ugm/liter, initially) was thus increased by 1 ugm/liter per zinc-65 dose in the first experiment versus 0.1 ugm/liter in the other four. Other experiment variations included: (1) a 4 uc overdose was inadvertently

TABLE 4

SUMMARY OF ALGAE UPTAKE EXPERIMENTS

Experiment Number	Algae	Temperature Range (°C.)	pH Range	Algae Obtained (grams wet weight)	Per Cent Moisture	Activity (uc/gm dry weight)	Concentration Factor uc/gm dry weight / 7×10^{-5} uc/ml*
1	Nitzschia	14.9 - 18.0	8.05 - 9.60	21.4758	93.9	1.13	16,150
	Carteria	14.9 - 18.0	8.15 - 9.70	19.6424	91.6	1.24	17,700
2	Nitzschia	15.0 - 21.0	7.85 - 9.70	26.4802	93.5	2.48	35,500**
	Carteria	15.0 - 21.0	8.05 - 9.75	35.5265	91.1	0.91	13,000
3	Nitzschia	15.5 - 18.5	8.40 - 9.65	44.2564	95.4	0.75	10,700
	Carteria	15.5 - 18.5	8.45 - 9.80	30.2836	91.1	1.19	17,000
4	Nitzschia	15.0 - 19.5	8.30 - 9.40	40.0657	94.1	0.93	13,300
	Carteria	15.0 - 19.5	8.50 - 9.55	43.8759	93.8	1.13	16,150
5	Nitzschia	16.0 - 26.0	8.40 - 9.70	49.4963	94.5	0.88	12,600
	Carteria	16.0 - 26.0	8.50 - 9.90	48.1563	94.4	1.10	15,700

*Initial activity of medium.

**Four uc overdose inadvertently added to culture flask.

added to the Nitzschia culture in the second experiment, resulting in a concentration factor of 35,500 which precluded use of the algae in subsequent feeding experiments; and (2) the culture room temperature increased approximately 6°C. in experiment 5 due to equipment malfunction.

The pH gradually increased as CO_2 was consumed, and at 9.4 to 9.9 cells of both species began to settle and adhere to the culture flasks. This pH change was reproducible enough that culture progress could have been monitored by pH rather than cell count. Cultures required from three to five days to reach a maximum cell count, Nitzschia maximums being 10 to 20 times those of Carteria. The total zinc concentration of the culture medium was 79 ppb for each experiment.

Discussion

Although concentration factors for both species of algae were approximately the same (excluding experiment number 2), caution is necessary in interpretation of the absolute values of these factors as well as their similarity. The method of calculating the factors produces an arbitrary value, dependent on the zinc-65 concentration of the medium used as the divisor.

Tables 11 through 15 (Appendix 3) show that percentages of total radioactivity associated with algae are constantly high (70-100 per cent, in most cases) regardless of the amount of zinc-65 added to the culture medium. The 95 per cent confidence limit of these percentages is ± 20 per cent, and related data suggest that in reality all the zinc-65 was removed from the medium by each algae. Thus, if the concentration factors were based on an average of the initial and final zinc-65 concentrations of the medium, their value would be doubled. The initial concentration was used in these calculations to simulate the condition in which a continuous source of zinc-65 of constant concentration is available. In Figures 8 and 9 ranges of the amount of zinc-65 per algal cell and the number of cells per milliliter are plotted versus time. These graphs show that as the algal cell count increases, the radioactivity per cell decreases by a factor of 10 or more from a maximum reached after the first dose, suggesting a potential uptake capacity for zinc-65 much higher than that represented by the final radioactivity per cell. That the cells' activity at harvest is only a fraction of their total capacity is suggested by the maximum concentration of zinc-65 per Nitzschia cell

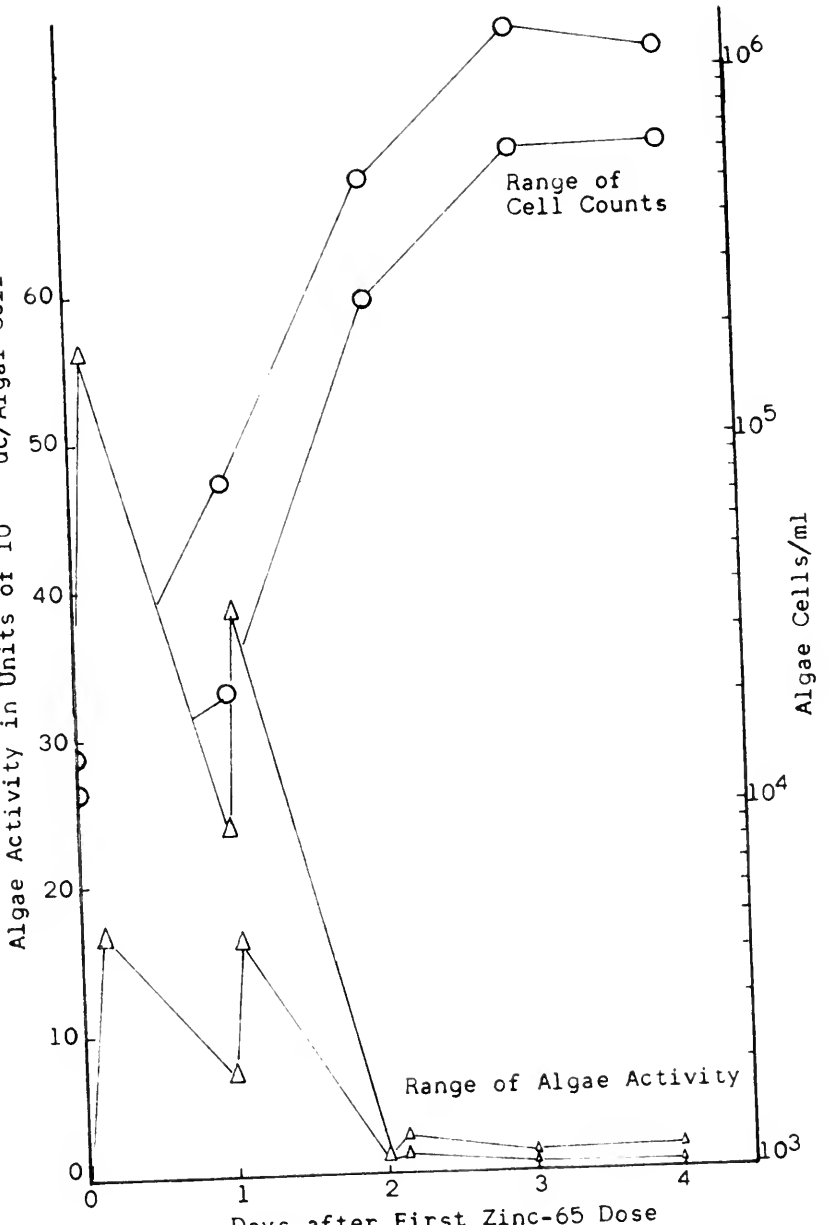

FIGURE 8

SUMMARY OF NITZSCHIA CLOSTERIUM
ACTIVITY AND CELL COUNT

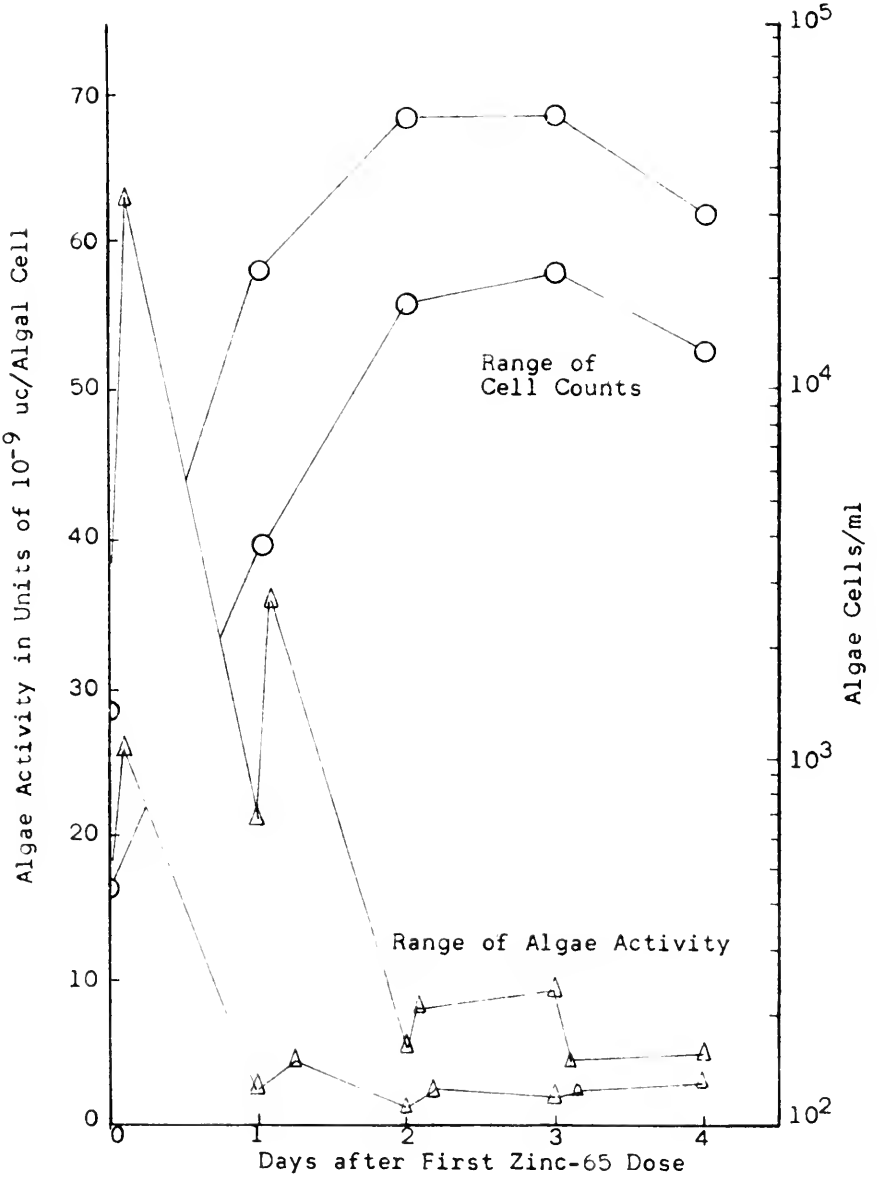

FIGURE 9

SUMMARY OF CARTERIA SP. ACTIVITY AND CELL COUNT

after the overdose in experiment 2 (Table 12). There is no evidence that even this excess of zinc-65 approached the limit of algal uptake potential. Consequently, comparison of the concentration factors for the two algae or the magnitude of the factors with others reported in the literature is not meaningful inasmuch as unavailability of zinc limited their uptake rather than species characteristics. However, this limiting phenomenon is of importance to this research because it indicates the probability of zinc-65 in a waste discharge stream becoming rapidly and quantitatively associated with the marine algae population of the discharge area. This would be particularly true during times of nutrient enrichment of the receiving water and the accompanying probability of algae blooms.

The increase in zinc concentration of the culture medium due to using a low specific activity zinc-65 source was too small to have a measurable effect on algae uptake.

Mullet

Results

Four parameters determined by the mullet feeding phase of the research are important to MPC theory.

They are: (1) a mathematical model characterizing zinc-65 uptake, (2) zinc-65 elimination rates for mullet, (3) the distribution of accumulated zinc-65 among mullet tissues, and (4) the maximum concentration of zinc-65 in the mullet. Results are discussed in that order.

Mathematical model. Zinc-65 increase in the mullet with time is tabulated in Table 16 (Appendix 4) and Figures 10 and 11 show these data for two representative fish from each treatment (Nitzschia and Carteria). These four curves typify the uptake patterns exhibited by the mullet. The goodness of fit of these uptake data to equation 9 ($I_{rf} = I_{rf\ max}(1 - e^{-(K+B_f)t})$ (see Chapters I and II) was determined by plotting $\ln(I_{rf\ max} - I_{rf})$ versus time for each fish. It will be recalled from Chapter II that by taking natural logarithms of both sides of the above equation, it can be converted to a linear equation of the form

$$\ln(I_{rf\ max} - I_{rf} = \ln I_{rf\ max} - (K + B_f)t .$$

Therefore, if equation 9 characterizes the uptake, a plot of $\ln(I_{rf\ max} - I_{rf})$ versus time will be a straight line. Maximum uptakes ($I_{rf\ max}$) were calculated by averaging the radioactivity per fish

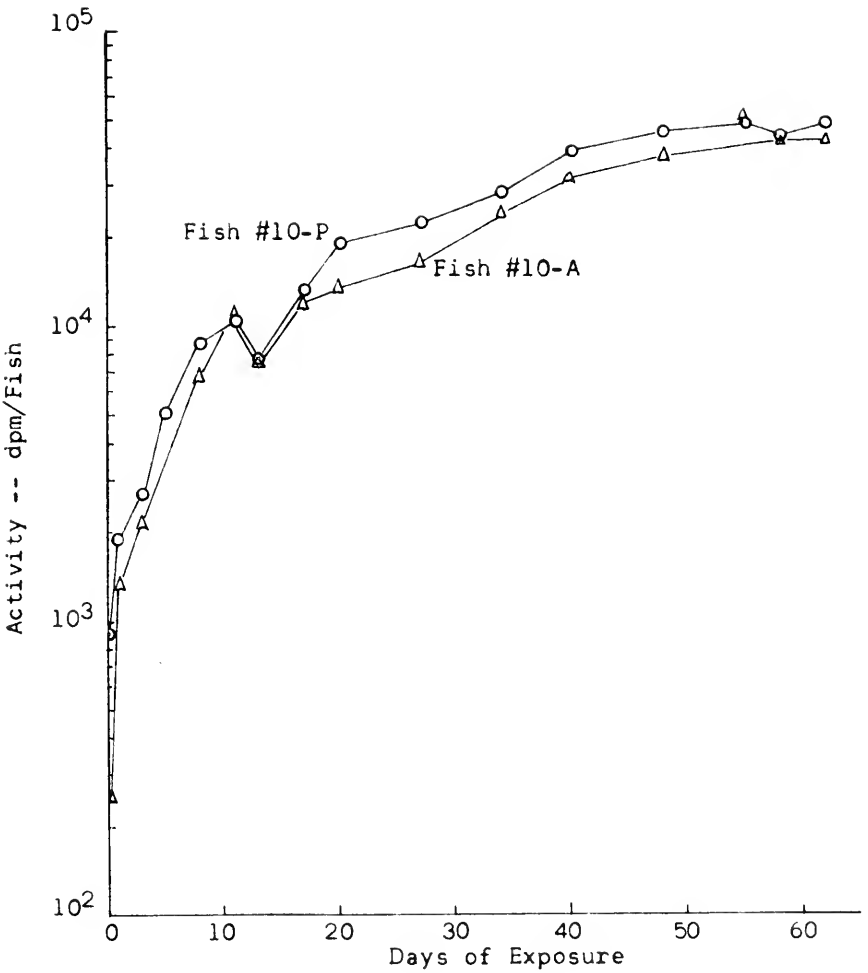

FIGURE 10

INCREASE IN MULLET ACTIVITY WITH TIME
NITZSCHIA TREATMENT

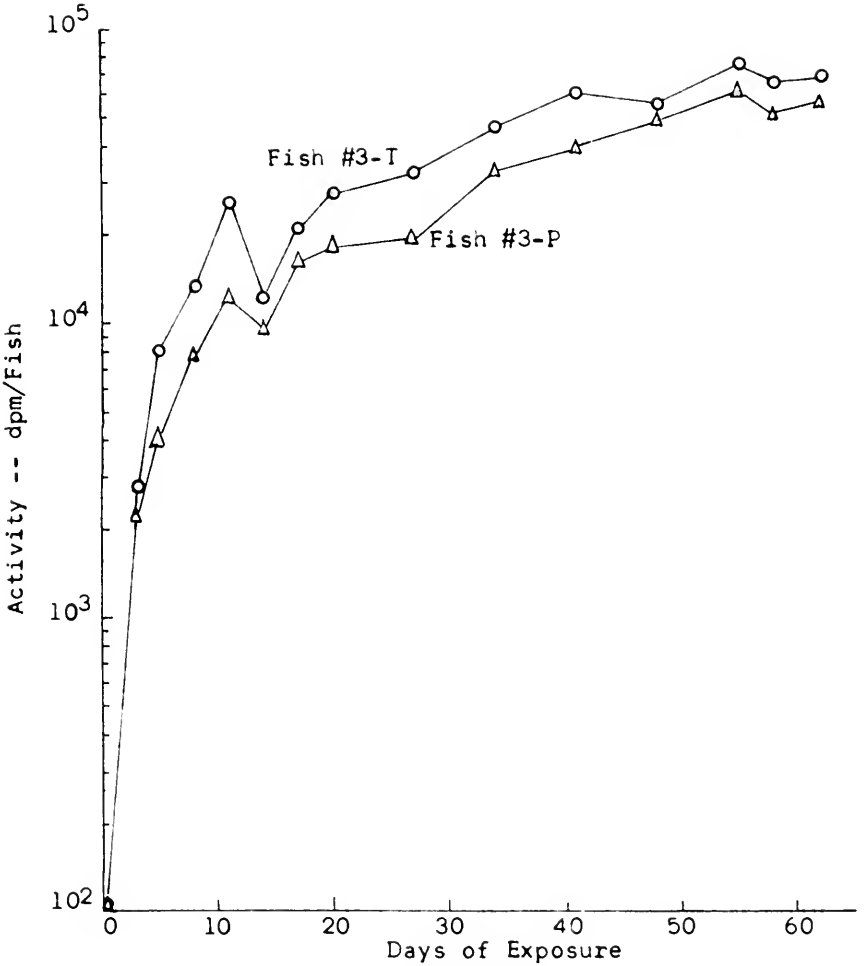

FIGURE 11

INCREASE IN MULLET ACTIVITY WITH TIME
CARTERIA TREATMENT

as it began to fluctuate around the maximum. The resultant graphs for the fish of Figures 10 and 11 are shown in Figure 12. The curves show that for the first 30 to 40 days, the data satisfy equation 9, but as equilibrium is approached, the straight line relation is no longer valid. Other fish show similar results, and the slopes of the regression lines and corresponding effective half-lives for each fish, except number 7-T, are presented in Table 5. The regressions were determined for the first 34 days, thus excluding the portion of uptake not in agreement with equation 9. Corresponding slope values may therefore be biased downward and it is necessary to know if the time of maximum uptake can be predicted from the equation. Using the average of the slopes of Table 5, -0.024, the time to reach 90 per cent of equilibrium ($I/I_{max} = 0.9$) is calculated to be 96 days. Figure 10 shows that the apparent maximum is reached in 55 to 60 days; so, either estimated slopes are biased, or the maximum is not reached in 60 days as indicated. The latter possibility is the most appealing, because the errors involved in counting live fish make it difficult to accurately measure the close approach to equilibrium. Although the

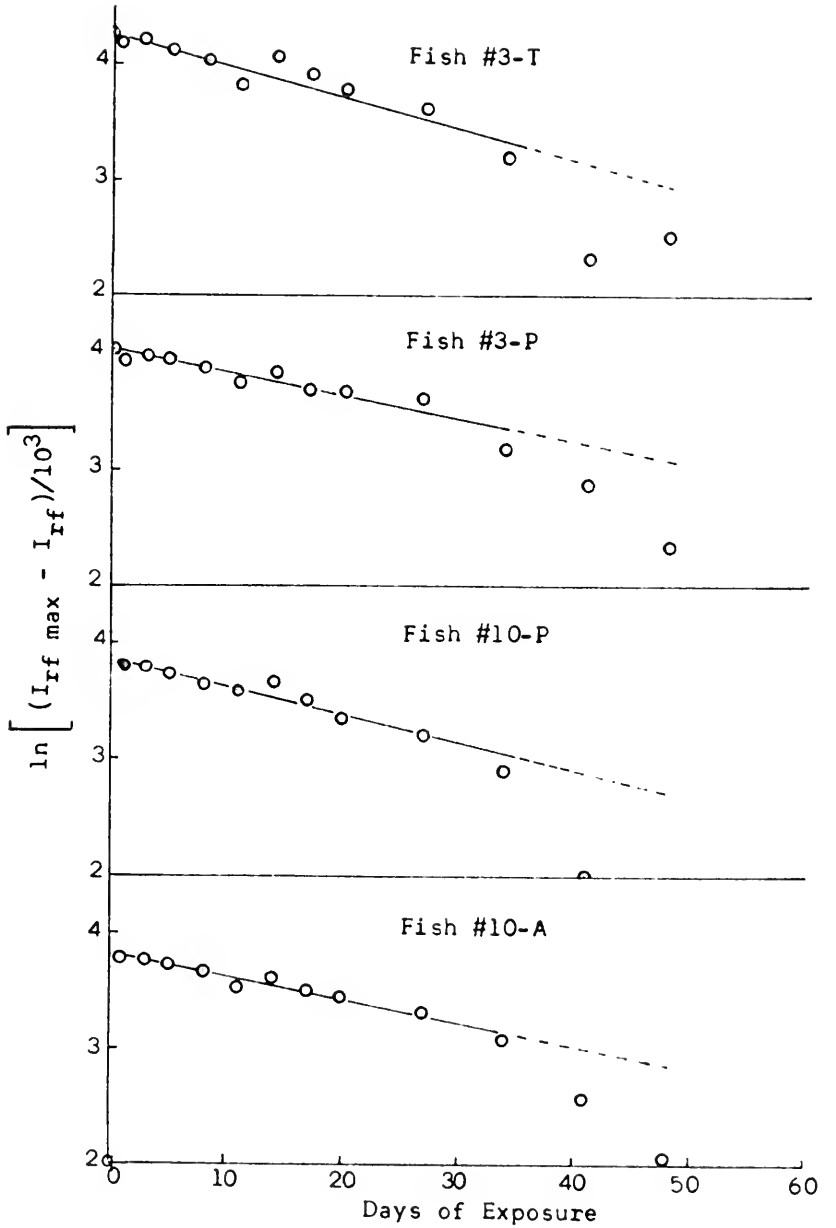

FIGURE 12

VERIFICATION OF MULLET UPTAKE EQUATION

TABLE 5

ZINC-65 ELIMINATION DATA FOR UPTAKE FISH

Fish No.	Algae Fed	b	$T_{1/2}$
1-T	Nitzschia	- 0.021	33.0
7-T	Nitzschia	--	--
10-A	Nitzschia	- 0.019	36.5
10-P	Nitzschia	- 0.025	27.8
11-T	Nitzschia	- 0.027	25.7
2-A	Carteria	- 0.036	19.3
2-T	Carteria	- 0.021	33.0
3-P	Carteria	- 0.020	34.7
3-T	Carteria	- 0.027	25.7
8-A	Carteria	- 0.029	23.9
8-T	Carteria	- 0.026	26.7
9-P	Carteria	- 0.027	25.7
9-T	Carteria	- 0.020	34.7

excretion mechanism of the fish may change near equilibrium (invalidating equation 9), it is concluded that the equation accurately describes the mullet uptake.

Elimination rates. Effective half-lives determined from the uptake equation 9 are presented in Table 5. From the average of these values, 29 days, the average biological half-life of zinc in mullet is calculated to be 33 days, indicating that metabolic turnover of zinc-65 is approximately 8 times faster than the physical decay (245 day half-life). No dependence of elimination rate on algae used as food is evident, and although not indicated in Table 5, the rates did not show any trend with mullet size.

The accuracy of the elimination rates determined from the uptake equation was evaluated by direct observation of the radioactivity decrease in mullet. Seven[1] mullet of size comparable to the uptake fish were fed both radioactive algae for 33 days and then fed non-radioactive food and their decrease in radioactivity monitored for 21 days. This decrease should follow an equation of the form $A = A_0 e^{-(K+B_f)t}$ where A = radioactivity at any time, t; A_0 = initial radioactivity;

[1]Originally 4 for each algae, but one Carteria-fed fish died.

and K and B_f are elimination constants previously defined. Therefore, a linear plot of ln A versus time should give a straight line with slope equal to $-(K + B_f)$. The decrease of radioactivity in these fish is graphed on a semilog scale in Figure 13 and the calculated slopes and half-lives tabulated in Table 6. The average of effective half-lives is 71 days corresponding to a biological half-life of 100 days. Approximately a two-fold difference exists, therefore, between the elimination rates measured by the two different methods, a result partly due to inability to prevent reingestion of excreted zinc-65 by the decay fish in the closed system. The aquarium water was monitored routinely for zinc-65 during the investigation and none was found. However, this did not exclude the possibility of the fish taking up the nuclide by eating the slimes along the aquarium walls. These slimes were monitored at the end of the experiment and found to contain approximately 3×10^{-3} uc zinc-65/gm wet weight. Also, the time to reach equilibrium (230 days) predicted from the 71 day half-life is so far from the observed time that this half-life must be suspect. Nevertheless, the values are not different in order of magnitude, and define a range within which the true value probably falls.

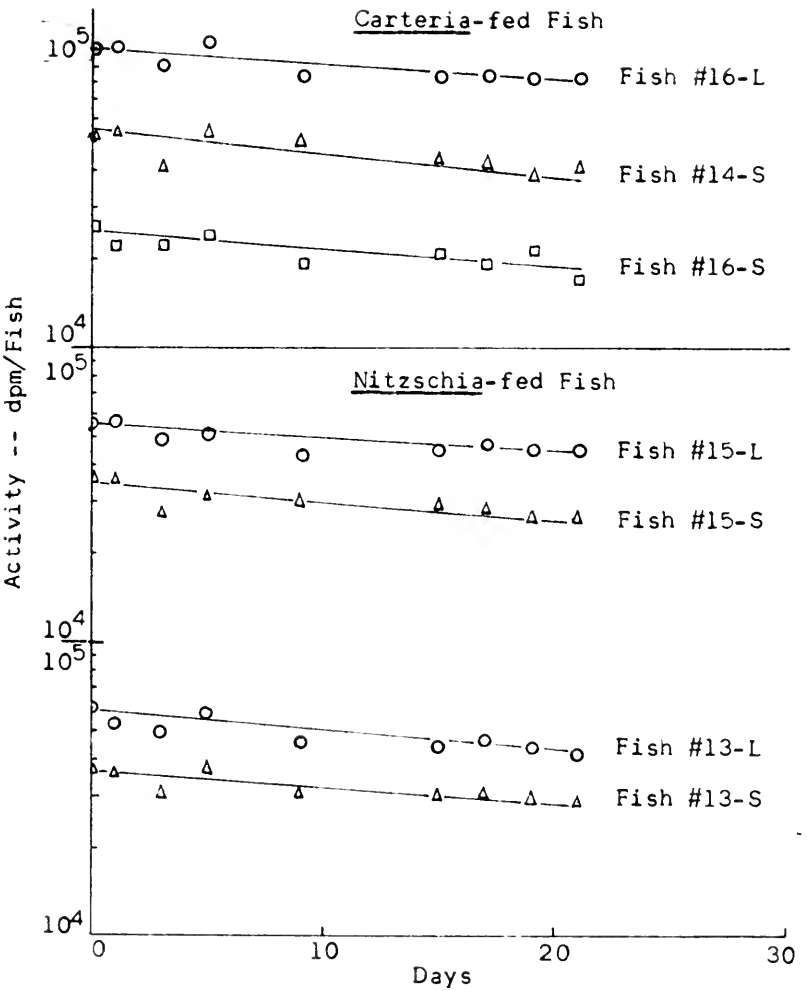

FIGURE 13

DECREASE OF MULLET ACTIVITY WITH TIME

TABLE 6

ZINC-65 ELIMINATION DATA FOR DECAY FISH

Fish No.	Algae Fed	b	$T_{1/2}$
13-L	Nitzschia	-0.011	63
13-S	Nitzschia	-0.008	87
15-L	Nitzschia	-0.010	69
15-S	Nitzschia	-0.009	77
14-S	Carteria	-0.010	69
16-L	Carteria	-0.011	63
16-S	Carteria	-0.010	69

Tissue distribution. Uptake fish were sacrificed at their maximum activity and decay fish after 21 days of zinc-65 elimination. The total zinc-65 and the zinc-65 concentration per gram of dry weight were determined in 12 tissues (referring to ingested food as a tissue) of each fish. Each tissue was then ranked in relation to the other 11 by these two parameters. The radioactivity and ranking data are included in Tables 18 through 29 of Appendix 4. To evaluate trends in these individual data, the ranks (excluding control fish) were totaled and the totals represented by bar graphs in Figures 14 and 15. These graphs show the cumulative ranks in decreasing order as follows:

	Total Activity		Concentration: Activity/gm	
Rank	Uptake	Decay	Uptake	Decay
1	Bone	Bone	Eyes	Eyes
2	Skin	Skin	Liver	Skin
3	Scales	Scales	Alimentary Tract	Viscera
4	Muscle	Muscle	Skin	Alimentary Tract
5	Eyes	Eyes	Viscera	Liver
6	Alimentary Tract	Alimentary Tract	Heart	Scales
7	Gills	Gills	Spleen	Bone
8	Liver	Viscera	Scales	Gills
9	Ingested Food	Liver	Gills	Heart
10	Viscera	Ingested Food	Bone	Spleen
11	Spleen	Heart	Muscle	Muscle
12	Heart	Spleen		

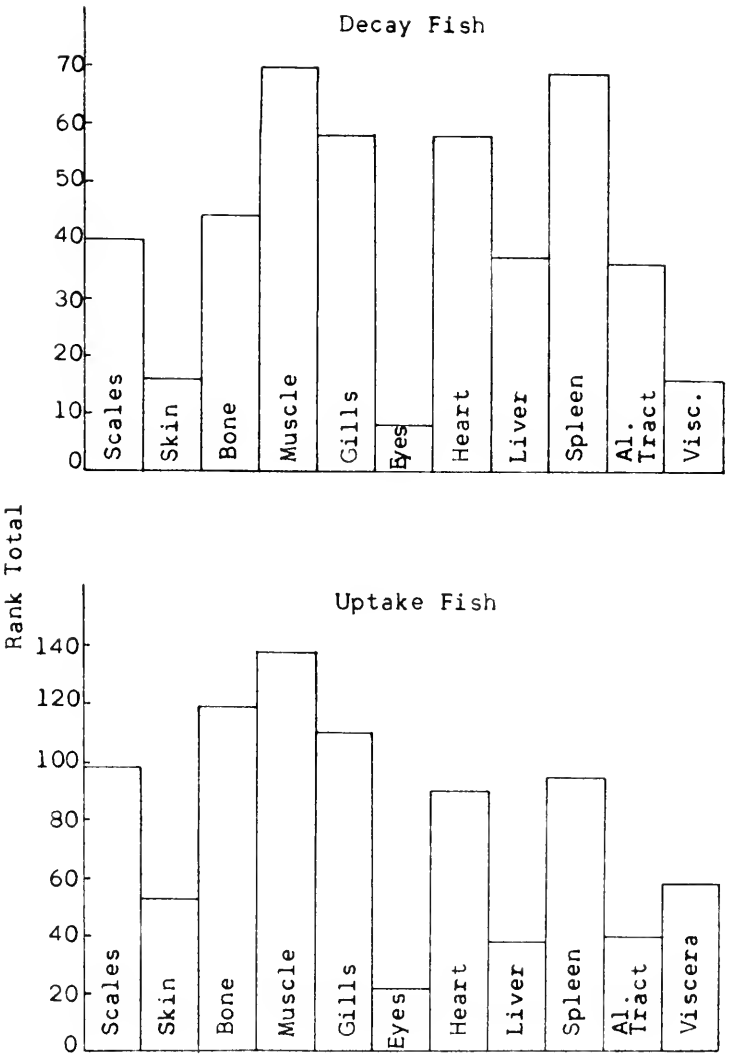

FIGURE 14

COMPARISON OF RANKS OF TISSUE CONCENTRATION OF ZINC-65 FOR UPTAKE AND DECAY FISH

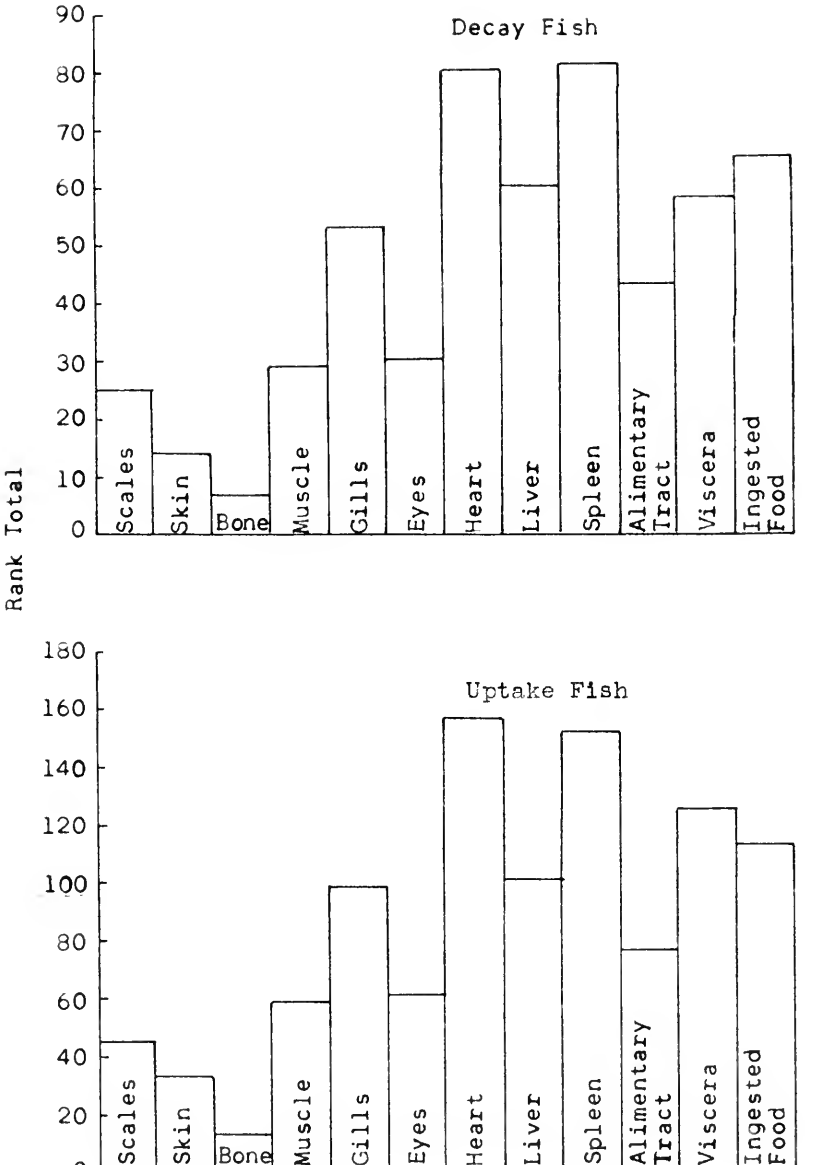

FIGURE 15

COMPARISON OF TOTAL ZINC-65 CONTENT OF TISSUE FOR UPTAKE AND DECAY FISH

Two facts stand out in these rankings: (1) the eyes consistently concentrate zinc-65 more than any other tissue, and (2) the bone, skin, scales, and muscle contain the majority of the zinc-65 present in the fish, although these concentrations, particularly those of bone and muscle, are low. In addition, excretion patterns are evident in the concentration rankings. For example, the liver, second in uptake fish, is fifth in the decay fish, indicating rapid loss of zinc-65 from this organ. Conversely, bone increases in rank to seventh as a result of slow elimination. Using these data, it may be seen that on a relative basis the liver, alimentary tract, heart, and spleen lose zinc-65 rapidly whereas bone, skin, viscera, scales, and gills retain it. It is of interest to note that ingested food does not account for much of the accumulated activity. Had this been an acute short-term exposure experiment, the reverse would have been true.

Maximum concentration. At the time the uptake fish were sacrificed, their total zinc-65 content was determined. This radioactivity, expressed as dpm/gram of dry weight, was used for statistical analyses of the effects of fish size and algae on the uptake. In

addition to the algae-fed mullet, eight mullet were fed nonradioactive fish food as a control. This was done to evaluate any uptake of excreted zinc-65 which was solubilized and available to all fish in addition to the zinc-65 in the algae diet. During the course of the experiment three of the fish in the Nitzschia treatment died, but all fish in the other treatments survived. The deaths of the Nitzschia fish resulted from the fish jumping from the aquaria and malfunction of the aquarium air supply, rather than as any result of the treatment itself.

Three separate statistical analyses were performed: (1) partitioning of the total error of the experiment into its components using duplicate radioactivity determinations on each fish; (2) measurement of the treatment effects by partitioning of the effects into two components; and (3) covariance analysis of the average of determinations using fish size as the covariate. The resultant analyses of variance and covariance for the three cases are shown in Tables 7, 8, and 9, and the basic data in Appendix 4 (Tables 30 and 31).

The analytical error analysis of variance (Table 7) was carried out to isolate instrumental error from

TABLE 7

UPTAKE FISH--
ANALYTICAL ERROR ANALYSIS OF VARIANCE

Source	df	Sum of Squares	Mean Square	F
Treatments	2	9337.76	4668.88	86.01**
Aquaria Within Treatments	9	488.56	54.28	
Fish Within Aquaria	9	1471.67	163.52	
Analyses on Fish	21	2.86	0.14	
Total	41	11,300.85		

TABLE 8

STATISTICAL ANALYSIS OF MAXIMUM ACTIVITIES OF UPTAKE FISH

Analysis of Variance					Partition of Treatment Effects				
Source	df	Sum of Squares	Mean Square	F	Source	df	Sum of Squares	Mean Square	F
Treatments	2	4668.88	2334.44	86.01**	Treatment	2	4668.88	2334.44	--
Aquaria Within Treatments	9	244.27	27.14	--	Control vs. *Nitzschia* + *Carteria*	1	3927.86	3927.86	144.72**
Fish Within Aquaria	9	735.84	81.76	--	*Nitzschia* vs. *Carteria*	1	741.02	741.02	27.30**
Total	20	5648.99							

TABLE 8 (continued)

Analysis of Covariance

Source	df	Sum of Products Σx^2(length)	Σxy	Σy^2(activity)	df	Σdyx^2	Mean Square
Total (Fish)	12	8.27	−83.02	1719.44			
Treatments	1	0.71	−23.04	741.02			
Aquaria Within Treatments	6	2.05	− 0.05	243.63	5	243.63	48.73
Fish Within Aquaria	5	5.51	−59.93	734.79	4	82.96	
Treatments + Aquaria Within Treatments	7	2.76	−23.09	984.65	6	791.48	
Treatments Adjusted					1	547.85	547.85
							F = 11.2**
							$F_{.025}$ = 10.01

TABLE 9

HOMOGENEITY OF TREATMENT REGRESSIONS

Treatment	df	Σx^2 (length)	Σxy	Σy^2 (activity)	b	Deviations from Regression df	Deviations from Regression $d_{y.x}^2$
Nitzschia	4	1.03	−2.96	168.30	−2.98	3	159.79
Carteria	7	6.56	−57.02	810.14	−8.69	6	314.52
						9	474.31
Nitzschia + Carteria	11	7.59	−59.98	978.44	−11.67		504.45

$$F = \frac{(504.45 - 474.31)}{(474.31)/9}$$

$$= 0.57 \quad \text{not significant}$$

the error due to fish variation and aquarium differences. That instrumental variations are a very small fraction of total error can be seen by comparison of the analyses on fish mean square (0.14) with the other two error components, fish within aquaria (163.52) and aquaria within treatments (54.28). Experiments of this nature gain very little, therefore, by duplication of radioactivity determinations. In future investigations of this kind, time and money would be more wisely spent by increasing the number of fish used. In view of the insignificance of the instrument error, determinations per fish were averaged for the remainder of the computations.

Using average values without covariance (Table 8), a highly significant treatment effect is observed ($F = 86.01$)[1] due to the magnitude of the control fish uptake being so small versus the Nitzschia and Carteria fish uptake (Table 30). To get a true appraisal of the effect of the two algae treatments, this treatment effect is partitioned into a comparison of the control fish versus the test fish and a comparison of the two

[1]The denominator of the F ratio is the aquaria within treatment mean square. However, it may be seen that this mean square is less than the fish within aquaria value, indicating that the error component due to aquaria is zero (Steel and Torrie, 1960). The fish within aquaria mean square could, therefore, be used as the divisor, but significant effects are still observed.

test fish. This partitioning shows that the treatment effect is mainly a result of the small control uptake ($F = 144.72$), but a significant difference between the Nitzschia and Carteria treatments is still indicated ($F = 27.30$). However, before this significance can be attributed to actual difference in mullet uptake from the two algae, it is necessary to evaluate the possibility that the difference may have been due to different size fish in the two treatments. This is accomplished by the covariance analysis (Tables 8 and 9). By this technique, the fraction of the total treatment variation attributable to the regression of activity on fish length (age) is removed from the treatment effect before significance testing. The use of covariance analysis is based on the assumption that the regressions of radioactivity on length are the same for all treatments. To test this assumption, the data of Table 30 are graphed in Figure 16. A strong dependence of activity on length for the test fish is indicated, but little if any dependence is suggested by the control fish. Based on this observation, the control fish were not included in the covariance analysis. The homogeneity of the two treatment regressions was tested as suggested by Steel and Torrie (1960), and no significant difference could be detected ($F = 0.57$,

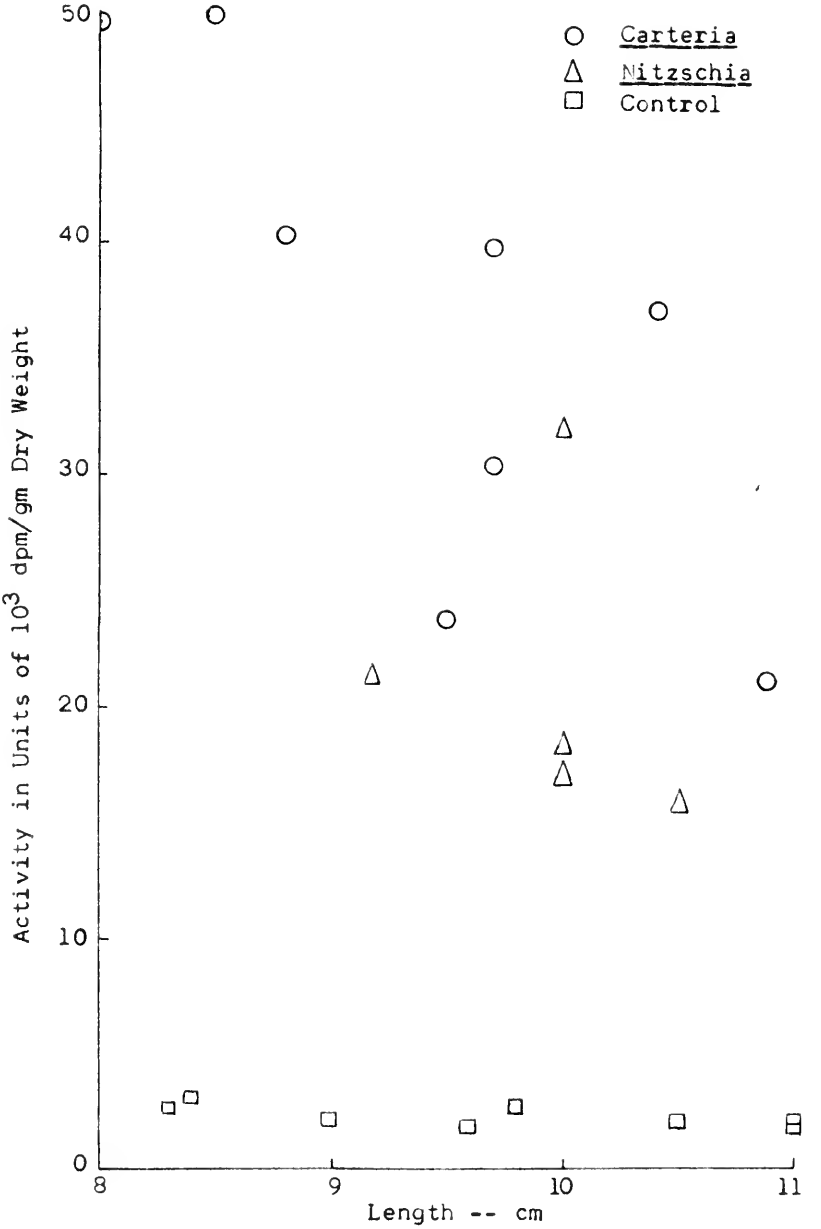

FIGURE 16

VARIATION IN MULLET ACTIVITY WITH LENGTH

Table 9). The subsequent covariance analysis is presented in Table 8 and shows that even after removal of the size effect, the maximum uptake by Carteria-fed mullet is greater than for those fed Nitzschia ($F = 11.2$). This difference is particularly significant considering the small numbers of fish in the test.

To be accurate, the means of the two treatments should be adjusted for regression before comparison. However, the average size of the mullet in both treatments was comparable; so comparison of unadjusted means is satisfactory for the purpose of this research. The means of both size and length and the standard deviation for each treatment with the corresponding concentration factors are presented in the following table.

TABLE 10

SUMMARY OF ZINC-65 CONCENTRATIONS IN MULLET

Measurement	Carteria Treatment	Nitzschia Treatment
Mean Activity (uc/gm dry weight)	$(16.40 \pm 1.94) \times 10^{-3}$	$(9.45 \pm 2.43) \times 10^{-3}$
Mean Length (cm)	9.4 ± 0.4	9.9 ± 0.5
Food Activity (uc/gm dry weight)	$(92.8 \pm 1.7) \times 10^{-3}$	$(69.1 \pm 1.7) \times 10^{-3}$
Concentration Factor		
Food	0.18	0.14
Water	230	135

Two facts in this table are particularly important: (1) the zinc-65 concentration in mullet is reduced, compared to the food, by a factor of 6 to 7, and (2) within a one standard deviation error, the difference in concentration between Nitzschia and Carteria fed mullet is explainable by the difference in zinc-65 concentrations of the two fish foods.

The maximum concentrations of Table 10 correspond to the fraction $\frac{a\ I_{rf_1}\ M}{K + B_{f_2}}$ from equation 12, Chapter II, where (a) is the fraction of ingested zinc-65 assimilated by the mullet, (I_{rf_1}) is the concentration of zinc-65 in the ingested food, (M) is the weight of food eaten per day, and (K) and (B_f) are the physical and biological constants of zinc-65 in mullet. Although the research was not designed for this purpose, the data of Table 10 permit an estimation of the fraction (a) by assuming that each fish ate 0.5 gm of food per day. Using the average elimination constant determined by the uptake equation, (a) for the Carteria treatment is calculated to be 0.008 and for the Nitzschia 0.007, suggesting that the mullet assimilate approximately 1 per cent of the zinc-65 they ingest.

Discussion

Mathematical model. The general equation $I_{rf} = I_{rf\ max}\ (1 - e^{-(K+B_f)t})$ has been shown to describe

the uptake of zinc-65 by mullet up to at least 75 per cent of the maximum uptake. Beyond this point, experimental data no longer fit the equation, but it is expected that this deviation, in part, is due to inability to measure accurately the approach to equilibrium. The only other explanation would be that near the equilibrium point a different function governs the rate of accumulation or elimination of the zinc. Such a change is possible, but without *a priori* knowledge, it would not be expected.

Assuming its validity, the value of the model is considerable. With it estimates can be made of the important parameters, $I_{rf\ max}$ and B_f, without the necessity of conducting lengthy experiments. For example, if zinc-65 contamination of a marine area is known to have occurred and B_f is known, $I_{rf\ max}$ can be estimated by a single measurement of I_{rf} at a known time after the contamination. On the other hand, B_f values can be estimated by making two determinations of I_f at known times, and dividing the resulting equations. $I_{rf\ max}$ is thus eliminated so B_f can be determined. A third use is for predicting the time required to reach a given fraction of the maximum activity. By the equation, equilibrium is only attained after infinite time, but practically, 90 or 95 per cent is a suitable approximation.

Perhaps the most important aspect of the model concerning this research is that its applicability substantiates the MPC theory which uses the model as a basis. The ICRP (1960) points out that a power function model gives a good fit to uptake of some very long-lived nuclides, but they assumed equation 9 was valid for all other nuclides. Their assumption seems justified at least for zinc-65 in mullet, and some evidence exists in the literature for its applicability to other nuclides in other organisms. Davis and Foster (1958), for example, found that Caddis fly larva uptake of phosphorous-32 from labeled Spirogyra followed this equation, and Rice's (1963) work with zinc-65 and brine shrimp has already been cited. If the model can be shown to be valid for the common sea food organisms, and the equation parameters determined for each, MPC calculations will be possible without need of highly conservative assumptions.

For mullet under the conditions studied, the equation parameters have been estimated to be:

B_f = 0.007 to 0.021 $days^{-1}$

I_{max} = 9.45×10^{-3} to 16.45×10^{-3} uc/gm dry weight

t at approximate I_{max} = 60 to 90 days

Further, the additional mullet parameter which governs I_{max}, (a) = fraction of ingested zinc assimilated, was estimated to be 1 per cent or slightly less.

The larger B_f estimate determined from the uptake fish is probably nearer the true value because of known inaccuracies in the decay fish technique. I_{max} is a variable dependent both on mullet characteristics and the method of feeding used, and although I_{max} varied with algal species, the variation probably reflected different concentrations of zinc in the algae food rather than differences in the mode of uptake. Another parameter affecting I_{max} is the total zinc content of the fish food. Both the Carteria and Nitzschia foods were made at the same time from identical raw materials, but polarographic analysis showed the Carteria food to contain 41 ppm zinc versus 78 ppm in the Nitzschia food. The smaller amount in Carteria food would tend to increase the concentration factor in Carteria-fed mullet, which lends further support to the conclusion that there is no difference in the mode of uptake from the two algae.

The value of I_{max} was found to be dependent on fish size, decreasing rapidly as size increased. This rate of decrease would have to slow considerably as the mullet approach adult size (> 1 foot) if the larger

fish were to accumulate any activity. Consequently, a quantitative estimate of this rate is not appropriate to this research; it is sufficient to know that edible size mullet would have a lower concentration of zinc-65 at their maximum uptake.

The estimated value of 1 per cent for the fraction (a) is very approximate, because the research was not designed to measure this variable. However, this value is within the range of similar values estimated for the human body organs (.004 per cent to 10 per cent; ICRP, 1960), suggesting that it is a valid estimate. Knowledge of the value of this fraction for adult food organisms along with the elimination constants, K and B_f, and some simple assumptions, would permit estimation of $I_{rf\ max}$ values for various zinc-65 exposure levels.

Based on the prediction of equation 9, the observed time of 60 days to reach I_{max} is probably somewhat short, 90 to 100 days being a more likely estimate. Although the size differential of the mullet was not great enough to bring out any trend of B_f values with size, work reported in Chapter II indicates that B_f should decrease with size, resulting in longer times to reach I_{max} with larger fish.

The activity accumulated in relatively large amounts in those tissues normally processed for

consumption, bone, skin, and muscle, is of public health significance. Obviously, these fish must be considered as a sea food subject to zinc-65 contamination. On the other hand, the concentration of zinc-65 in these tissues is low so that the resulting radiation dose to the body per gram of ingested food is low.

Zinc-65 concentrated in the eyes of the mullet makes these organs useful for indicating the presence of zinc-65 in the fish and also in the marine environment as Joyner (1962) has suggested. However, unless it were known that a mullet had reached its maximum zinc uptake when sampled, prediction of the quantity of contamination present in the environment would not be possible from a simple determination of the eye radioactivity. On the other hand, the relative concentrations of zinc in various organs could be used as an indication of whether the fish was accumulating zinc or not. Thus, if liver concentration exceeded skin concentration appreciably, the conclusion could be drawn with some confidence that this fish was in an uptake phase and that zinc-65 was continuously available in the fish's environment. If the skin was greater, it could similarly be concluded that the fish was showing a net loss of zinc and probably did

not have a continuous supply available. Based on this reasoning, an economical monitoring program for a marine area can be visualized. Mullet could be sampled periodically and only their eyes checked for radioactivity. If activity was present, its distribution in the fish could then be determined, and a decision made on a more extensive sampling program. In those instances where sampling and analytical determinations are expensive or difficult, this type monitoring program would be important, because it provides a maximum of information for a minimum of expense and effort.

MPC Calculations

As stated in Chapter I, the underlying purpose of calculating the value of the parameters affecting MPC determinations is to evaluate the existing MPC for zinc-65 in sea water (7×10^{-9} uc/ml). This MPC is a conservative estimate and may be unduly restrictive. In order to obtain a meaningful evaluation, the conditions affecting the parameters should be such that the values determined represent the most hazardous situation which could possibly prevail under natural conditions. It is therefore necessary to consider how far the laboratory system deviates from these conditions.

In general, the greatest hazard to humans from zinc-65 contaminated sea food will occur when the hold-up time[1] in the marine food chain is shortest resulting in the least radioactive decay. This corresponds to high B_f values. In this research, nonedible size mullet were used, but the work of other investigators has indicated that as the size of an organism increases, its elimination rate (B_f) decreases. Therefore, the determined value of B_f is undoubtedly greater than the value for an adult mullet. The other criterion affecting the MPC is the maximum concentration achieved in the mullet ($I_{rf\ max}$) and it was shown that this maximum decreases with fish size. Again, therefore, the determined values are larger than would be expected in adult mullet. It was seen that the algae concentration factors for zinc-65 were probably much lower than might be possible if more zinc were available. However, the initial zinc-65 concentration of the culture medium was 10,000 times greater than the current MPC for zinc-65, and it is unlikely that under natural conditions concentrations this high would be encountered. It wouldn't be expected, therefore, that algae under natural conditions would

[1]Hold-up time is used to refer to the time required for organisms to attain their maximum accumulation of zinc-65.

accumulate more zinc-65 than these laboratory cultures. The total zinc[1] concentration in the water could also affect the mullet uptake of zinc-65; during the uptake experiment the concentration in the aquarium water averaged 29.8 ppb which closely approximates that of normal sea water. The total zinc concentrations of the Carteria and Nitzschia fish foods were 40.6 and 77 ppm, respectively. Parker (1962) found that algae (natural mullet food) contained 60 to 89 ppm of zinc, so the artificial fish food is not appreciably different in total zinc content from natural mullet food. It cannot be said with certainty that the two algae chosen, Nitzschia closterium and Carteria sp., are the highest algae concentrators of zinc-65. However, they removed all the available zinc-65 from the culture medium and Morgan (1961) from a study of six species found Nitzschia closterium and the Chlamydomonadaceae to concentrate zinc-65 more than any other alga investigated. As previously pointed out, the application of elimination constants to these algal cells must be considered with caution. Conservatively, however, the time for these cells to divide is comparable to the effective half-life in higher organisms, because their division rate limited

[1]Total zinc refers to radioactive plus non-radioactive isotopes.

maximum uptake in this study. This time of division was approximately 0.5 days for both algae during their uptake of zinc-65, corresponding to a B_{f_1} value of 0.51 day^{-1}.

From these considerations, it appears that the MPC parameter values determined in this laboratory system represent, if anything, a more hazardous condition than might exist in a natural mullet-algae food chain. Therefore, calculation of an MPC with these parameters should give a figure which is not overly optimistic.

It was pointed out in Chapter I that the NAS-NRC (1960) calculated MPC's by first assuming an organ other than the GI tract as critical and second assuming the GI tract as a critical organ. Using the determined values of $B_{f_2} = 0.021$, $B_{f_1} = 0.51$, $B_{\text{human body}} = 0.0007$ (ICRP, 1960), and $K = 0.0028$, the corresponding calculations are:

Case I. GI tract not critical.

$$MPC = I_{re} \approx \frac{I_{rb}}{I_{nb}} I_{ne} \left[1 + \frac{K^3 + K^2(B + B_{f_1} + B_{f_2})}{BB_{f_1}B_{f_2}} + \frac{K(BB_{f_1} + BB_{f_2} + B_{f_1}B_{f_2})}{BB_{f_1}B_{f_2}} \right]$$

K and B are small compared to B_{f_1} and B_{f_2} so the equation reduces to

$$MPC \simeq \frac{I_{rb}}{I_{nb}} I_{ne} \left[1 + \frac{K^2(B_{f_1} + B_{f_2}) + K(B_{f_1}B_{f_2})}{BB_{f_1}B_{f_2}} \right]$$

$$\simeq \frac{I_{rb}}{I_{nb}} I_{ne} \left[1 + \frac{34.3 \times 10^{-6}}{7.5 \times 10^{-6}} \right]$$

$$\simeq \frac{I_{rb}}{I_{nb}} I_{ne} \left[5.6 \right]$$

$$\simeq 5 \frac{I_{rb}}{I_{nb}} I_{ne}$$

Inasmuch as the NAS-NRC (1960) used the fraction $\frac{I_{rb}}{I_{nb}} I_{ne}$ to determine the MPC, their value is lower approximately by a factor of five than the value calculated from these experimental data.

Case II. GI tract critical.

$$MPC = I_{re} \simeq \frac{10(MPC)_w}{F} \left[1 + \frac{K^2 + K(B_{f_1} + B_{f_2})}{B_{f_1}B_{f_2}} \right]$$

Again the equation reduces to

$$MPC \simeq \frac{10(MPC)_w}{F} \left[1 + \frac{K(B_{f_1} + B_{f_2})}{B_{f_1}B_{f_2}} \right]$$

$$\simeq \frac{10(MPC)_w}{F} \left[1 + \frac{0.0028(0.51 + 0.02)}{(0.51)(0.02)} \right]$$

$$\simeq \frac{10(MPC)_w}{F} \left[1.14 \right]$$

$$\simeq \frac{10(MPC)_w}{F}$$

The NAS-NRC used the value $\frac{10(MPC)_w}{F}$ to determine their MPC, so the elimination constants do not appreciably change this determination. They used a value of 5000 for F, compared to 230 determined in this research, but found that Case I resulted in the lowest value, so it is not pertinent to consider this difference in F values.

It is apparent from these calculations that the conservative MPC calculated by the NAS-NRC may err on the side of safety. The degree of this deviation should be substantiated by additional food chain studies, preferably on adult sea food organisms. Until this can be done, and the recommended value raised accordingly, the existing MPC is the best estimate that can be used, but its provisional nature should be recognized.

CHAPTER V

SUMMARY

A laboratory experiment was conducted to study the transfer of the induced radionuclide, zinc-65, through a two-step marine food chain consisting of the algae, Nitzschia closterium and Carteria sp., and mullet of the genus Mugil. The purpose of the study was to obtain values for the parameters utilized in MPC calculations so that the existing recommended MPC for zinc-65 in sea water could be evaluated in terms of a natural food chain supplying food to man. Findings included:

1) Both species of algae rapidly took up all the zinc-65 from a medium whose concentration was 10,000 times the MPC.

2) The Carteria concentration of zinc-65 in relation to the culture medium concentration was slightly higher than in Nitzschia.

3) Juvenile mullet which were fed an artificial food containing the zinc-65 labeled algae reached an apparent maximum uptake in 55 to 60 days.

4) The mullet's accumulation of zinc-65 was accurately described by an equation of the form

$$I_{rf} = I_{rf\ max}\left[1 - e^{-(K + B_f)t}\right]$$

for approximately 75 per cent of the uptake period.

5) The maximum concentration of zinc-65 in the mullet was significantly greater in those fish fed Carteria food. However, this difference was probably a reflection of higher concentrations of zinc-65 in Carteria and lower total zinc concentrations in Carteria food rather than some difference in the mode of uptake from the two species.

6) The mullet's maximum zinc-65 concentration was a function of fish size, decreasing rapidly as size increased.

7) Biological elimination constants for zinc in mullet ranged from 0.021 to 0.011, the higher number probably being nearer the true value.

8) Zinc was concentrated in the eyes of the mullet, but the bone, scales, skin, and flesh accounted for the majority of the total zinc-65 present in the fish.

9) Zinc was lost most rapidly from the liver, alimentary tract, heart, and spleen, whereas bone, skin, viscera, scales, and gills retained it.

10) Economical monitoring programs could be devised to detect zinc-65 contamination of marine areas by measuring zinc-65 concentration in fish eyes, followed by determination of relative tissue concentrations when indicated.

11) The fraction of ingested zinc which is assimilated by mullet tissue was estimated to be 1 per cent.

12) Although zinc is highly concentrated by algae, the mullet do not concentrate but rather discriminate against the algae concentration by a factor of 10 or more.

13) The MPC calculated with the determined parameters would be approximately 5 times higher than the current recommended value.

Recommendations

Investigations should be carried out to determine the MPC parameters (biological elimination constants and assimilation fractions) for additional sea food organisms, and the applicability of the uptake equation to these organisms be evaluated. If such research consistently indicates that the current MPC for zinc-65 is too low, it should be raised accordingly.

APPENDICES

APPENDIX 1

FORMULA FOR RICE'S CULTURE MEDIUM

Three nutrient solutions are used in preparation of Rice's medium. They are:

Solution A.

KNO_3 . . . 20.2 grams/100 cc distilled water

Solution B.

$MgSO_4$ . . . . . 4 grams

$CaCl_2$ . . . . . 4 grams

$Fe(NH_4)(SO_4)_2$ . . . . . 1 gram

HCl (conc.) . . . . . 2 cc

Dilute this mixture to 100 cc with distilled water.

Solution C.

KH_2PO_4 . . 1.53 grams/100 cc distilled water

The medium is prepared by adding 0.55 cc of Solution A and 0.5 cc of Solution B to one liter of sea water. This mixture is autoclaved at 121°C. for 15 minutes, allowed to cool for 24 hours, and 0.5 cc of Solution C added. The medium is then ready for use.

APPENDIX 2

ZINC ANALYTICAL PROCEDURES

Dithizone Technique

Sea water samples were initially analyzed by a dithizone extraction procedure used at the Bureau of Commercial Fisheries, Radiobiological Laboratory in Beaufort, North Carolina. Only the general procedure is outlined here; details are available in mimeographed form from the laboratory.

Procedure

1) To 200 ml of water add 10 ml concentrated HNO_3, take to dryness, and drive off all acid.
2) Take residue into solution with 5 ml 0.1 N HCl (redistilled) and 100 ml deionized water and allow to dissolve for 12 hours.
3) Transfer solution to a 500 ml separatory funnel. To another separatory funnel add 5 ml 0.1 N HCl and 5 ugm of zinc in solution. This is the standard.
4) Bring each solution to 200 ml with deionized water.

5) Each solution is then buffered at pH 5.5 and interfering agents masked by addition of a potassium sodium tartrate solution and a solution of sodium thiosulfate, sodium acetate, and potassium cyanide.
6) The solutions are then extracted with 0.002 per cent dithizone in CCl_4 and the absorbance of the extract read at 530 mu with pure 0.002 per cent dithizone as the reference.
7) A blank is carried through the entire procedure and its absorbance subtracted from the sample and standard.
8) The zinc content of the sample is determined by the formula

$$\text{ugm zinc in sample} = \frac{\text{ugm zinc in standard}}{\text{corrected absorbance of standard}} \times \text{corrected absorbance of sample}$$

Polarographic Technique

The majority of the stable zinc analyses were performed by polarographic stripping analysis using a Sargent Model FS recording polarograph. The instrumental procedures used were adapted from unpublished work by Professor George B. Morgan and Mrs. Keith Gubbins of the University of Florida. Specific operating

instructions for the polarograph are available from the manufacturer, and only the general techniques used are reported here.

Procedure

1) Prepare liquid samples for polarography by digestion with concentrated HNO_3 three times. For a 200 ml sample, three 10 ml portions of HNO_3 are used. Solid samples are ashed at 600°C. for 4 hours and then digested once with 10 ml of HNO_3.
2) To the oxidized sample, add 100 ml of 0.5 M KSCN and stir until dissolution is complete.
3) Adjust the pH of the solution to 7 with redistilled HCl. (This step is essential.)
4) On the polarograph, electrolyze this solution at -1.2 volts for 5 minutes.
5) By stripping technique, obtain the current voltage curve for the zinc taken into the hanging mercury drop.
6) Obtain the height of the curve maximum and read the zinc content from a standard curve.
7) Prepare the standard curve using artificial

sea water[1] which is taken through the same preparation procedure as the sample. Current-voltage curves are determined after adding incremental amounts of a standard zinc solution to the artificial sea water residue (taken up in 100 ml KSCN) and a curve of peak height versus zinc added is plotted.

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[1]Artificial sea water formula (adapted from Sverdrup, et al., 1946):

Quantities per 2 liters of deionized water.

$NaCl$	46.952 grams
$MgCl_2 \cdot 6H_2O$	21.220 grams
Na_2SO_4	7.834 grams
$CaCl_2$	2.920 grams
KCl	1.328 grams
$NaHCO_3$	0.384 grams

APPENDIX 3

TABLE 11

ALGAE UPTAKE -- EXPERIMENT NO. 1

Date	Time	Room Temperature (°C.)	pH	Cells/ml	Activity (uc/ml) Algae	Activity (uc/ml) Algae + Medium	Per Cent Activity Associated with Algae	uc Activity/ Algal Cell	Time of Zinc-65 Dose
					Nitzschia				
2- 7-65	1030	17.0	8.05	13,800	0	0	0	0	
	1330			13,800	2.28×10^{-5}	7.10×10^{-5}	32.0	16.5×10^{-10}	1200 hrs
2- 8-65	1300	14.9	8.05	21,000	4.90×10^{-5}	7.85×10^{-5}	62.5	23.4×10^{-10}	
	1430			21,000	8.15×10^{-5}	15.3×10^{-5}	53.4	38.8×10^{-10}	1330 hrs
2- 9-65	1030	16.5	8.40	520,000	11.8×10^{-5}	14.5×10^{-5}	81.3	2.27×10^{-10}	
	1330			520,000	16.5×10^{-5}	21.0×10^{-5}	78.6	3.17×10^{-10}	1205 hrs
2-10-65	1000	15.8	8.87	585,000	16.9×10^{-5}	22.4×10^{-5}	75.5	2.89×10^{-10}	
	1400			585,000	23.3×10^{-5}	29.9×10^{-5}	78.0	3.98×10^{-10}	1200 hrs
2-11-65	1000	16.0	9.60	617,000	21.9×10^{-5}	25.4×10^{-5}	86.2	3.55×10^{-10}	
					Carteria				
2- 8-65	1800	14.9	8.15	1,420	0	0	0	0	
	1900			1,420	3.75×10^{-5}	7.15×10^{-5}	52.5	26.4×10^{-9}	1815 hrs
2- 9-65	1445	20.5	8.41	20,200	4.83×10^{-5}	6.86×10^{-5}	70.5	2.40×10^{-9}	
	1930			20,200	8.64×10^{-5}	12.9×10^{-5}	67.0	4.28×10^{-9}	1715 hrs
2-10-65	1645	18.0	9.55	39,000	6.72×10^{-5}	10.5×10^{-5}	64.0	1.72×10^{-9}	
	2030			39,000	9.45×10^{-5}	13.1×10^{-5}	72.2	2.42×10^{-9}	1745 hrs
2-11-65	1630	17.0	9.70	21,000	4.10×10^{-5}	8.10×10^{-5}	50.5	1.94×10^{-9}	

TABLE 12

ALGAE UPTAKE -- EXPERIMENT NO. 2

Date	Time	Room Temperature (°C.)	pH	Cells/ml	Activity (uc/ml) Algae	Activity (uc/ml) Algae + Medium	Per Cent Activity Associated with Algae	uc Activity/ Algal Cell	Time of Zinc-65 Dose
					Nitzschia				
2-19-65	0930	16.0	7.85	8,200	0	0	0	0	
	1315			8,200	14.7×10^{-5}	6.35×10^{-5}	100	180×10^{-10}	1135 hrs
2-20-65	1000	16.0	8.15	21,700	4.80×10^{-5}	6.00×10^{-5}	79.6	22.2×10^{-10}	
	1245			21,700	28.8×10^{-5}	37.2×10^{-5}	77.3	133×10^{-10}	1130*hrs
2-21-65	1045	15.0	8.50	201,000	33.2×10^{-5}	38.6×10^{-5}	85.2	16.4×10^{-10}	
	1250			201,000	40.5×10^{-5}	43.2×10^{-5}	92.7	20.2×10^{-10}	1145 hrs
2-22-65	1030	19.0	9.20	1,360,000	45.8×10^{-5}	44.2×10^{-5}	100	3.36×10^{-10}	
	1230			1,360,000	48.7×10^{-5}	52.3×10^{-5}	100	3.58×10^{-10}	1135 hrs
2-23-65	1000	21.0	9.70	1,185,000	34.4×10^{-5}	40.4×10^{-5}	86.6	2.90×10^{-10}	
					Carteria				
2-19-65	1930	15.0	8.05	840	0	0	0	0	
	2230			840	5.40×10^{-5}	5.80×10^{-5}	94.3	64.2×10^{-9}	2035 hrs
2-20-65	2030	19.0	8.30	3,900	5.40×10^{-5}	5.00×10^{-5}	100	13.9×10^{-9}	
	**			3,900	9.80×10^{-5}	11.0×10^{-5}	88.0	25.1×10^{-9}	2100 hrs
2-21-65	2000	16.5	8.80	17,700	8.60×10^{-5}	12.6×10^{-5}	62.4	4.86×10^{-9}	
	2230			17,700	13.3×10^{-5}	17.5×10^{-5}	75.5	7.51×10^{-9}	2100 hrs
2-22-65	2000	18.5	9.75	13,400	12.5×10^{-5}	15.1×10^{-5}	82.0	9.34×10^{-9}	

*Four uc overdose inadvertently added to culture flask.
**Exact time not recorded; approximate time 2130.

TABLE 13

ALGAE UPTAKE -- EXPERIMENT NO. 3

Date	Time	Room Temperature (°C.)	pH	Cells/ml	Activity (uc/ml) Algae	Algae + Medium	Per Cent Activity Associated with Algae	uc Activity/ Algal Cell	Time of Zinc-65 Dose
					Nitzschia				
2-28-65	1030	17.0	8.40	12,000	0	0	0	0	
	1200			12,000	5.50×10^{-5}	6.50×10^{-5}	84.6	45.8×10^{-10}	1100 hrs
3- 1-65	1000	18.5	8.50	35,900	5.20×10^{-5}	6.30×10^{-5}	82.4	14.5×10^{-10}	
	1230			35,900	10.5×10^{-5}	13.2×10^{-5}	79.5	29.2×10^{-10}	1120 hrs
3- 2-65	1000	16.0	8.85	445,000	12.3×10^{-5}	13.7×10^{-5}	89.7	2.76×10^{-10}	
	1500			445,000	20.0×10^{-5}	22.3×10^{-5}	89.7	4.50×10^{-10}	1100 hrs
3- 3-65	1000	16.5	9.30	1,340,000	17.8×10^{-5}	21.2×10^{-5}	84.0	1.33×10^{-10}	
	1205			1,340,000	21.5×10^{-5}	26.6×10^{-5}	80.8	1.60×10^{-10}	1105 hrs
3- 4-65	0930	16.0	9.65	1,040,000	24.3×10^{-5}	26.4×10^{-5}	92.2	2.34×10^{-10}	
					Carteria				
2-28-65	2100	15.5	8.45	1,190	0	0	0	0	
	*			1,190	5.50×10^{-5}	7.20×10^{-5}	76.4	46.2×10^{-9}	2200 hrs
3- 1-65	2155	16.0	8.55	5,850	10.1×10^{-5}	5.90×10^{-5}	100	17.3×10^{-9}	
	2400			5,850	10.9×10^{-5}	13.7×10^{-5}	79.5	18.7×10^{-9}	2300 hrs
3- 2-65	2100	18.0	9.05	31,000	11.5×10^{-5}	13.5×10^{-5}	85.0	3.71×10^{-9}	
	2325			31,000	19.7×10^{-5}	20.2×10^{-5}	97.5	6.35×10^{-9}	2200 hrs
3- 3-65	2030	16.5	9.80	55,600	9.60×10^{-5}	13.4×10^{-5}	71.6	1.74×10^{-9}	
	2325			55,600	11.5×10^{-5}	16.6×10^{-5}	69.2	2.06×10^{-9}	2210 hrs
3- 4-65	1045	16.0	9.80	27,300	8.10×10^{-5}	11.6×10^{-5}	69.9	2.98×10^{-9}	

*Exact time not recorded; approximate time 2200.

TABLE 14

ALGAE UPTAKE -- EXPERIMENT NO. 4

Date	Time	Room Temperature (°C.)	pH	Cells/ml	Activity (uc/ml) Algae	Activity (uc/ml) Algae + Medium	Per Cent Activity Associated with Algae	uc Activity/ Algal Cell	Time of Zinc-65 Dose
					Nitzschia				
3- 8-65	1115	15.0	8.30	17,100	0	0	0	0	
	1215			17,100	9.50×10^{-5}	6.20×10^{-5}	100	55.6×10^{-10}	1120 hrs
3- 9-65	1015	15.0	8.45	80,300	5.60×10^{-5}	6.50×10^{-5}	86.0	6.98×10^{-10}	
	1115			80,300	12.9×10^{-5}	13.2×10^{-5}	97.8	16.0×10^{-10}	1115 hrs
3-10-65	0930	15.0	8.90	249,000	9.30×10^{-5}	11.8×10^{-5}	78.8	3.75×10^{-10}	
	1210			249,000	13.1×10^{-5}	20.8×10^{-5}	62.9	5.25×10^{-10}	1110 hrs
3-11-65	1030	19.0	9.40	970,000	14.5×10^{-5}	17.5×10^{-5}	82.8	1.50×10^{-10}	
	1205			970,000	20.9×10^{-5}	25.1×10^{-5}	83.2	2.16×10^{-10}	1130 hrs
3-12-65	1000	14.5	9.40	1,240,000	15.9×10^{-5}	18.6×10^{-5}	85.5	1.28×10^{-10}	
					Carteria				
3- 8-65	2030	19.5	8.50	436	0	0	0	0	
	2205			436	10.0×10^{-5}	5.40×10^{-5}	100	230×10^{-9}	2100 hrs
3- 9-65	2030	19.5	8.40	5,960	6.80×10^{-5}	7.50×10^{-5}	90.5	11.4×10^{-9}	
	2230			5,960	12.6×10^{-5}	12.8×10^{-5}	98.5	21.1×10^{-9}	2105 hrs
3-10-65	2030	15.0	8.95	17,200	9.00×10^{-5}	14.4×10^{-5}	62.5	5.24×10^{-9}	
	2210			17,200	13.7×10^{-5}	20.4×10^{-5}	67.2	7.94×10^{-9}	2110 hrs
3-11-65	2030	14.5	9.50	39,600	13.0×10^{-5}	12.5×10^{-5}	100	3.29×10^{-9}	
	*			39,600	15.0×10^{-5}	20.4×10^{-5}	73.5	3.78×10^{-9}	2100 hrs
3-12-65	1100	14.5	9.55	12,400	20.9×10^{-5}	18.3×10^{-5}	100	16.9×10^{-9}	

*Exact time not recorded; approximate time 2130.

TABLE 15

ALGAE UPTAKE -- EXPERIMENT NO. 5

Date	Time	Room Temperature (°C.)	pH	Cells/ml	Activity (uc/ml) Algae	Activity (uc/ml) Algae + Medium	Per Cent Activity Associated with Algae	uc Activity/ Algal Cell	Time of Zinc-65 Dose
					Nitzschia				
3-16-65	0945	16.0	8.40	13,500	0	0	0	0	
	1120			13,500	5.40×10^{-5}	6.70×10^{-5}	80.5	40.0×10^{-10}	1020 hrs
3-17-65	0930	16.0	8.40	50,000	8.60×10^{-5}	6.20×10^{-5}	100	17.2×10^{-10}	
	1130			50,000	14.1×10^{-5}	13.0×10^{-5}	100	28.1×10^{-10}	1020 hrs
3-18-65	0945	24.0	8.92	417,000	10.7×10^{-5}	11.6×10^{-5}	92.2	2.58×10^{-10}	
	1130			417,000	16.2×10^{-5}	19.0×10^{-5}	85.2	3.90×10^{-10}	1020 hrs
3-19-65	0930	17.0	9.65	650,000	19.1×10^{-5}	18.3×10^{-5}	100	2.94×10^{-10}	
	1130			650,000	27.8×10^{-5}	26.8×10^{-5}	100	4.28×10^{-10}	1020 hrs
3-20-65	1030	16.0	9.70	675,000	26.8×10^{-5}	26.6×10^{-5}	100	3.98×10^{-10}	
					Carteria				
3-16-65	2030	21.0	8.50	1,150	0	0	0	0	
	2210			1,150	5.60×10^{-5}	7.00×10^{-5}	80.0	48.7×10^{-9}	2110 hrs
3-17-65	2030	25.0	8.48	3,720	7.70×10^{-5}	7.10×10^{-5}	100	20.6×10^{-9}	
	2230			3,720	13.3×10^{-5}	12.6×10^{-5}	100	35.8×10^{-9}	2110 hrs
3-18-65	2045	26.0	9.40	53,400	6.90×10^{-5}	14.7×10^{-5}	47.0	1.29×10^{-9}	
	2220			53,400	17.3×10^{-5}	17.9×10^{-5}	96.7	3.24×10^{-9}	2110 hrs
3-19-65	2000	16.0	9.90	31,600	7.30×10^{-5}	9.30×10^{-5}	78.5	2.31×10^{-9}	
	2130			31,600	13.7×10^{-5}	16.7×10^{-5}	80.2	4.34×10^{-9}	2030 hrs
3-20-65	1130	16.0	*	28,500	13.0×10^{-5}	14.0×10^{-5}	92.8	4.56×10^{-9}	

*Value not recorded.

APPENDIX 4

TABLE 16

UPTAKE FISH--INCREASE IN FISH RADIOACTIVITY WITH TIME

Disintegrations per Minute per Fish

Days After Initial Dose	Nitzschia				
	1-T	7-T	10-A	10-P	11-T
0	306	0	259	895	259
1	11,360	700	1,340	1,900	460
3	3,330	1,330	2,130	2,700	400
5	4,990	3,420	4,450	5,190	4,300
8	7,250	6,750	6,730	8,800	6,940
11	18,700	11,370	11,080	10,800	13,550
14	14,300	5,960	7,940	8,300	9,490
17	18,700	10,850	11,900	13,510	18,300
20	28,000	7,000	13,700	18,850	18,750
27	41,800	7,920	16,900	22,700	27,200
34	53,200	14,050	23,400	28,800	27,800
41	85,800	16,550	32,100	40,700	39,100
48	91,000	23,400	37,500	47,100	48,600
55	111,000	32,300	54,200	50,700	51,000
58	88,700	34,000	41,200	43,400	47,500
62	98,000	35,800	40,800	48,300	50,000
66		48,000			
75		45,300			
81		55,000			
83		59,900			
85		58,500			
87		62,500			

TABLE 16 (Continued)

Carteria							
2-A	2-T	3-P	3-T	8-A	8-T	9-P	9-T
495	0	0	0	307	660	448	212
9,780	10,900	5,980	5,200	1,300	1,020	2,300	380
3,940	5,550	2,205	2,800	3,370	760	1,560	760
7,770	12,300	4,060	8,350	8,440	2,260	14,400	2,060
12,200	18,910	7,950	13,650	12,480	7,550	16,750	4,770
24,800	31,100	13,450	25,400	29,000	17,100	31,400	12,900
18,500	20,200	9,500	12,500	19,050	9,740	26,400	8,700
26,000	27,000	15,750	20,400	22,900	15,350	27,900	13,280
29,100	31,700	17,100	27,500	32,400	20,000	31,400	20,400
42,900	36,100	18,900	32,600	44,000	26,600	43,600	30,500
57,100	42,800	32,000	46,000	51,000	36,200	47,800	35,600
61,800	60,800	38,000	60,400	56,700	38,700	71,300	45,300
62,100	72,800	45,600	58,100	78,500	58,500	77,200	66,500
78,500	82,600	62,000	76,500	93,300	62,300	85,800	64,800
70,000	75,600	50,500	63,000	73,600	56,500	78,600	63,500
73,000	80,000	56,000	72,400	73,000	58,600	74,100	71,000

TABLE 17

DECAY FISH--DECREASE IN RADIOACTIVITY WITH TIME

Disintegrations Per Minute Per Fish

Days After Starting Decay	Nitzschia				Carteria		
	13-L	13-S	15-L	15-S	14-S	16-L	16-S
0	59,700	36,500	55,000	35,500	53,400	106,300	26,300
1	52,300	35,100	56,000	35,200	54,200	107,000	22,500
3	49,500	29,600	48,300	27,000	40,700	90,400	22,700
5	58,200	37,500	50,600	31,000	56,400	109,500	23,700
9	46,200	29,400	43,500	29,200	50,000	85,800	19,800
15	44,400	30,300	46,000	29,300	43,500	87,800	21,300
17	47,100	31,000	47,100	28,000	42,100	86,500	19,400
19	44,500	29,100	43,700	26,800	37,500	80,000	21,900
21	42,200	28,600	44,400	26,800	44,100	80,000	17,200

TABLE 18

UPTAKE FISH -- TOTAL ACTIVITY PER TISSUE IN UNITS OF 10^{-5} uc

Fish #	Algae Fed	Scales	Skin	Bone	Flesh	Gills	Eyes	Heart	Liver	Spleen	Alimentary Tract	Viscera	Ingested Food	Total Activity
1-T	Nitzschia	516	517	711	288	116	243	7.19	150	12.0	211	46.4	109	2926.59
7-T	Nitzschia	184	387	522	242	57.0	152	3.00	90.0	2.80	121	38.7	42.1	1841.60
10-A	Nitzschia	206	210	362	169	85.4	129	7.42	78.4	2.54	113	26.9	67.6	1457.26
10-P	Nitzschia	242	206	297	182	68.9	135	5.94	108	7.84	115	39.6	50.9	1458.18
11-T	Nitzschia	208	299	426	140	75.6	98.0	3.20	76.5	5.96	135	66.8	50.3	1584.36
2-T	Carteria	385	366	834	222	127	256	7.60	77.7	7.16	171	54.9	70.1	2578.46
2-A	Carteria	359	345	567	270	112	259	13.9	129	8.46	183	43.0	127	2416.36
3-P	Carteria	227	247	586	151	80.5	153	9.55	102	0.65	120	23.2	73.3	1773.20
3-T	Carteria	401	375	810	220	107	237	7.81	95.0	4.34	127	37.8	108	2529.95
8-A	Carteria	438	356	631	193	108	231	6.60	80.5	7.26	129	38.3	102	2320.66
8-T	Carteria	320	315	610	175	87.3	149	1.70	53.2	--	131	68.0	61.5	1971.70
9-P	Carteria	398	366	678	141	150	215	6.60	64.5	5.72	196	126	101	2447.82
9-T	Carteria	246	444	656	258	105	201	10.7	60.2	2.60	124	39.4	79.6	2226.50
4-P	None	17.6	24.7	53.0	20.4	1.95	10.8	0.43	8.14	--	17.6	5.28	5.72	165.62
4-T	None	22.0	28.2	50.6	13.0	12.5	18.3	2.20	9.46	1.98	8.36	2.42	5.28	174.30
5-P	None	24.0	33.4	50.8	20.9	15.8	10.1	3.74	7.04	1.98	13.2	10.1	8.58	199.64
5-A	None	22.7	27.9	46.6	10.6	9.68	14.7	--	10.3	0.22	9.46	3.96	4.62	160.74
6-A	None	40.5	42.0	86.7	28.8	10.6	18.5	0.64	6.82	--	12.9	6.39	4.26	258.11
6-T	None	36.8	55.2	54.5	28.3	13.4	15.3	--	8.73	1.92	17.9	4.69	12.1	248.84
12-T	None	51.5	25.6	65.7	29.0	15.0	28.0	0.42	3.82	3.82	14.4	--	3.82	241.08
12-P	None	33.5	46.0	57.4	24.8	11.0	16.3	1.06	12.5	4.24	18.4	0.42	7.63	233.25

TABLE 19

UPTAKE FISH -- PERCENTAGE TOTAL ACTIVITY PER TISSUE

Fish #	Algae Fed	Scales	Skin	Bone	Flesh	Gills	Eyes	Heart	Liver	Spleen	Alimentary Tract	Viscera	Ingested Food
1-T	Nitzschia	17.63	17.66	24.29	9.84	3.96	8.30	0.25	5.12	0.41	7.21	1.58	3.72
7-T	Nitzschia	9.99	21.01	28.34	13.14	3.10	8.25	0.16	4.89	0.15	6.57	2.10	2.29
10-A	Nitzschia	14.14	14.41	24.84	11.60	5.86	8.85	0.51	5.38	0.17	7.75	1.85	4.64
10-P	Nitzschia	16.60	14.13	20.37	12.48	4.72	9.26	0.41	7.41	0.54	7.89	2.72	3.49
11-T	Nitzschia	13.13	18.87	26.89	8.84	4.77	6.18	0.20	4.83	0.38	8.52	4.22	3.17
2-T	Carteria	14.93	14.19	32.34	8.61	4.92	9.93	0.30	3.01	0.28	6.63	2.13	2.72
2-A	Carteria	14.86	14.28	23.46	11.17	4.64	10.72	0.58	5.34	0.35	7.57	1.78	5.26
3-P	Carteria	12.80	13.93	33.05	8.52	4.54	8.63	0.54	5.75	0.04	6.77	1.31	4.13
3-T	Carteria	15.85	14.82	32.02	8.70	4.23	9.37	0.31	3.76	0.17	5.02	1.49	4.27
8-A	Carteria	18.87	15.34	27.19	8.32	4.65	9.95	0.28	3.47	0.31	5.56	1.65	4.40
8-T	Carteria	16.23	15.98	30.94	8.88	4.43	7.56	0.09	2.70	--	6.64	3.45	3.12
9-P	Carteria	16.26	14.95	27.70	5.76	6.13	8.78	0.27	2.64	0.23	8.01	5.15	4.13
9-T	Carteria	11.05	19.94	29.46	11.59	4.72	9.03	0.48	2.70	0.12	5.57	1.77	3.58
4-P	None	10.63	14.91	32.00	12.32	1.18	6.52	0.26	4.92	--	10.63	3.19	3.45
4-T	None	12.62	16.18	29.03	7.46	7.17	10.50	1.26	5.43	1.14	4.80	1.39	3.03
5-P	None	12.02	16.73	25.45	10.47	7.91	5.06	1.87	3.53	0.99	6.61	5.06	4.30
5-A	None	14.12	17.36	28.99	6.59	6.02	9.14	--	6.41	0.14	5.88	2.46	2.87
6-A	None	15.69	16.27	33.59	11.16	4.11	7.17	0.24	2.64	--	5.00	2.48	1.65
6-T	None	14.79	22.18	21.90	11.37	5.38	6.15	--	3.51	0.77	7.19	1.88	4.86
12-T	None	21.36	10.62	27.25	12.03	6.22	11.61	0.17	1.58	1.58	5.97	--	1.58
12-P	None	14.36	19.72	24.61	10.63	4.72	6.99	0.45	5.36	1.82	7.89	0.18	3.27

TABLE 20

UPTAKE FISH -- DISTRIBUTION OF TOTAL TISSUE ACTIVITY

RANKED FROM HIGHEST TO LOWEST

Fish #	Algae Fed	Scales	Skin	Bone	Flesh	Gills	Eyes	Heart	Liver	Spleen	Alimentary Tract	Viscera	Ingested Food
1-T	Nitzschia	3	2	1	4	8	5	12	7	11	6	10	9
7-T	Nitzschia	4	2	1	3	8	5	11	7	12	6	10	9
10-A	Nitzschia	3	2	1	4	7	5	11	8	12	6	10	9
10-P	Nitzschia	2	3	1	4	8	5	12	7	11	6	10	9
11-T	Nitzschia	3	2	1	4	8	6	12	7	11	5	9	10
2-T	Carteria	2	3	1	5	7	4	11	8	12	6	10	9
2-A	Carteria	2	3	1	4	9	5	11	7	12	6	10	8
3-P	Carteria	3	2	1	5	8	4	11	7	12	6	10	9
3-T	Carteria	2	3	1	5	8	4	11	9	12	6	10	7
8-A	Carteria	2	3	1	5	7	4	12	9	11	6	10	8
8-T	Carteria	2	3	1	4	7	5	11	10	12	6	8	9
9-P	Carteria	2	3	1	7	6	4	11	10	12	5	8	9
9-T	Carteria	4	2	1	3	7	5	11	9	12	6	10	8
4-P	None	4*	2	1	3	10	6	11	7	12	5*	9	8
4-T	None	3	2	1	5	6	4	11	7	12	8	10	9
5-P	None	3	2	1	4	5	7*	11	10	12	6	8*	9
5-A	None	3	2	1	5	7	4	12	6	11	8	10	9
6-A	None	3	2	1	4	7	5	11	8	12	6	9	10
6-T	None	3	1	2	4	7	6	12	9	11	5	10	8
12-T	None	2	5	1	3	6	4	11	8*	9*	7	12	10*
12-P	None	3	2	1	4	8	6	11	7	10	5	12	9

*Equal ranking.

TABLE 21

DECAY FISH -- TOTAL ACTIVITY PER TISSUE IN UNITS OF 10^{-5} uc

Fish #	Algae Fed	Scales	Skin	Bone	Flesh	Gills	Eyes	Heart	Liver	Spleen	Alimentary Tract	Viscera	Ingested Food	Total Activity
13-L	Nitzschia	126	289	333	137	34.8	119	5.60	13.5	0.80	73.2	37.4	14.6	1183.90
13-S	Nitzschia	118	162	314	76.5	20.2	66.2	0.40	16.8	1.30	46.7	10.3	13.3	845.70
15-L	Nitzschia	128	252	302	151	37.0	138	3.40	24.5	--	86.5	25.6	33.5	1181.50
15-S	Nitzschia	118	137	276	59.1	22.2	63.4	0.60	17.9	0.40	41.4	20.7	2.50	759.20
14-S	Carteria	182	309	397	111	30.3	83.5	3.00	48.4	--	49.0	13.1	17.4	1243.70
16-L	Carteria	352	477	618	222	65.6	190	1.50	101	2.70	113	46.8	26.4	2216.00
16-S	Carteria	51.5	105	194	37.7	15.8	54.5	--	9.00	--	19.7	40.3	2.20	529.70

TABLE 22

DECAY FISH--PERCENTAGE TOTAL ACTIVITY PER TISSUE

Fish #	Algae Fed	Scales	Skin	Bone	Flesh	Gills	Eyes	Heart	Liver	Spleen	Alimentary Tract	Viscera	Ingested Food
13-L	Nitzschia	10.64	24.41	28.13	11.57	2.94	10.05	0.47	1.14	0.07	6.18	3.16	1.23
13-S	Nitzschia	13.95	19.16	37.13	9.04	2.39	7.83	0.05	1.99	0.15	5.52	1.22	1.57
15-L	Nitzschia	10.83	21.33	25.56	12.78	3.13	11.68	0.29	2.07	--	7.32	2.17	2.84
15-S	Nitzschia	15.54	18.04	36.35	7.78	2.92	8.35	0.08	2.36	0.05	5.45	2.73	0.33
14-S	Carteria	14.63	24.84	31.92	8.92	2.44	6.71	0.24	3.89	--	3.94	1.05	1.40
16-L	Carteria	15.88	21.52	27.89	10.02	2.96	8.57	0.07	4.56	0.12	5.10	2.11	1.19
16-S	Carteria	9.72	19.82	36.62	7.12	2.98	10.29	--	1.70	--	3.72	7.61	0.42

TABLE 23

DECAY FISH--DISTRIBUTION OF TOTAL TISSUE ACTIVITY
RANKED FROM HIGHEST TO LOWEST

Fish #	Algae Fed	Scales	Skin	Bone	Flesh	Gills	Eyes	Heart	Liver	Spleen	Alimentary Tract	Viscera	Ingested Food
13-L	Nitzschia	4	2	1	3	8	5	11	10	12	6	7	9
13-S	Nitzschia	3	2	1	4	7	5	12	8	11	6	10	9
15-L	Nitzschia	5	2	1	3	7	4	11	10	12	6	9	8
15-S	Nitzschia	3	2	1	5	7	4	11	9	12	6	8	10
14-S	Carteria	3	2	1	4	8	5	11	7	12	6	10	9
16-L	Carteria	3	2	1	4	8	5	12	7	11	6	9	10
16-S	Carteria	4	2	1	6	8	3	12*	9	11*	7	5	10

*Equal ranking.

TABLE 24

UPTAKE FISH--DISTRIBUTION OF TISSUE ACTIVITY IN UNITS OF 10^{-4} uc/GM DRY WEIGHT

Fish #	Algae Fed	Scales	Skin	Bone	Flesh	Gills
1-T	Nitzschia	114	214	54.7	35.0	60.6
7-T	Nitzschia	63.9	191	54.3	31.2	17.5
10-A	Nitzschia	42.4	103	35.2	17.4	58.9
10-P	Nitzschia	45.9	91.2	25.3	17.6	44.3
11-T	Nitzschia	53.1	116	32.9	16.6	37.9
2-T	Carteria	151	271	118	54.1	113
2-A	Carteria	50.8	130	35.7	27.4	50.0
3-P	Carteria	50.6	118	59.0	23.3	58.0
3-T	Carteria	124	184	106	37.2	90.8
8-A	Carteria	98.6	168	58.7	27.6	82.2
8-T	Carteria	140	304	104	48.7	111
9-P	Carteria	90.6	251	70.1	37.6	94.3
9-T	Carteria	55.7	297	72.1	44.0	112
4-P	None	3.74	13.5	5.22	3.28	1.46
4-T	None	8.29	15.2	7.22	2.86	12.4
5-P	None	7.08	14.9	5.60	3.11	10.8
5-A	None	9.28	19.9	6.45	1.81	9.97
6-A	None	6.72	15.3	5.57	2.18	5.04
6-T	None	5.89	11.4	3.46	2.29	5.90
12-T	None	9.15	11.7	4.42	2.22	7.08
12-P	None	6.77	18.0	5.63	2.48	6.98

TABLE 24 (Continued)

Eyes	Heart	Liver	Spleen	Alimentary Tract	Viscera	Ingested Food
339	54.9	151	92.3	187	121	325
228	52.6	183	104	142	104	151
189	137	242	57.7	148	96.1	223
198	57.7	220	157	113	154	166
115	50.7	163	142	136	130	173
517	146	273	188	284	227	354
269	158	219	121	155	136	262
269	121	260	23.2	158	160	262
412	170	432	121	209	193	344
366	143	230	77.2	175	170	323
363	44.7	375	--	224	165	311
379	77.6	250	159	172	237	284
330	151	256	--	186	189	329
18.6	7.11	12.0	--	21.6	22.0	17.1
41.8	30.6	20.0	86.1	14.4	26.0	29.3
16.8	48.6	15.2	43.0	13.0	23.8	22.9
32.1	--	24.9	6.11	17.9	21.0	28.7
21.8	10.6	19.8	--	9.51	25.4	16.0
16.5	--	19.5	34.3	17.1	5.29	26.5
32.2	9.42	24.6	46.6	13.6	--	15.7
22.1	18.0	36.0	94.2	17.4	4.04	19.2

TABLE 25

UPTAKE FISH--DISTRIBUTION OF TISSUE ACTIVITY* RANKED FROM HIGHEST TO LOWEST

Fish #	Algae Fed	Scales	Skin	Bone	Flesh	Gills	Eyes	Heart	Liver	Spleen	Alimentary Tract	Viscera	Ingested Food
1-T	Nitzschia	7	3	11	12	9	1	10	5	8	4	6	2
7-T	Nitzschia	8	2	9	11	12	1	10	3	7	5	6	4
10-A	Nitzschia	10	6	11	12	8	3	5	1	9	4	7	2
10-P	Nitzschia	9	7	12	11	10	2	8	1	4	6	5	3
11-T	Nitzschia	8	6	11	12	10	7	9	2	3	4	5	1
2-T	Carteria	8	5	10	12	11	1	9	4	7	3	6	2
2-A	Carteria	9	7	11	12	10	1	4	3	8	5	6	2
3-P	Carteria	10	7	8	12	9	1	6	3	11	5	4	2
3-T	Carteria	8	6	10	12	11	2	7	1	9	4	5	3
8-A	Carteria	8	6	11	12	9	1	7	3	10	4	5	2
8-T	Carteria	7	4	9	10	8	2	11	1	12	5	6	3
9-P	Carteria	9	3	11	12	8	1	10	4	7	6	5	2
9-T	Carteria	10	3	9	11	8	1	7	4	12	6	5	2
4-P	None	9	5	8	10	11	3	7	6	12	2	1	4
4-T	None	10	7	11	12	9	2	3	6	1	8	5	4
5-P	None	10	7	11	12	9	5	1	6	2	8	3	4
5-A	None	8	5	9	11	7	1	12	3	10	6	4	2
6-A	None	8	5	9	11	10	2	6	3	12	7	1	4
6-T	None	7**	6	10	11	8**	5	12	3	1	4	9	2
12-T	None	8	6	10	11	9	2	7	3	1	5	12	4
12-P	None	9	6**	10	12	8	3	5**	2	1	7	11	4

*Units of activity = uc/gm dry weight.
**Equal ranking.

TABLE 26

UPTAKE FISH--DISTRIBUTION OF TISSUE ACTIVITY* RANKED FROM HIGHEST TO LOWEST EXCLUDING INGESTED FOOD

Fish #	Algae Fed	Scales	Skin	Bone	Flesh	Gills	Eyes	Heart	Liver	Spleen	Alimentary Tract	Viscera
1-T	Nitzschia	6	2	10	11	8	1	9	4	7	3	5
7-T	Nitzschia	7	2	8	10	11	1	9	3	6	4	5
10-A	Nitzschia	9	5	10	11	7	2	4	1	8	3	6
10-P	Nitzschia	8	6	10	11	9	2	7	1	3	5	4
11-T	Nitzschia	7	5	10	11	9	6	8	1	2	3	4
2-T	Carteria	7	4	9	11	10	1	8	3	6	2	5
2-A	Carteria	8	6	10	11	9	1	3	2	7	4	5
3-P	Carteria	9	6	7	10	8	1	5	2	11	4	3
3-T	Carteria	7	5	9	11	10	2	6	1	8	3	4
8-A	Carteria	7	5	10	11	8	1	6	2	9	3	4
8-T	Carteria	6	3	8	9	7	2	10	1	11	4	5
9-P	Carteria	8	2	10	11	7	1	9	3	6	5	4
9-T	Carteria	9	2	8	10	7	1	6	3	11	5	4
4-P	None	8	4	7	9	10	3	6	5	11	2	1
4-T	None	9	6	10	11	8	2	3	5	1	7	4
5-P	None	9	6	10	11	8	4	1	5	2	7	3
5-A	None	7	4	8	10	6	1	11	2	9	5	3
6-A	None	7	4	8	10	9	2	5	3	11	6	1
6-T	None	7	5	9	10	6	4	11	2	1	3	8
12-T	None	7	5	9	10	8	2	6	3	1	4	11
12-P	None	8	5**	9	11	7	3	4**	2	1	6	10

* Units of Activity = uc/gm dry weight
** Equal ranking.

TABLE 27

DECAY FISH--DISTRIBUTION OF TISSUE ACTIVITY IN UNITS OF 10^{-4}/uc/gm DRY WEIGHT

Fish #	Algae Fed	Scales	Skin	Bone	Flesh	Gills
13-L	Nitzschia	23.28	128.73	26.06	10.30	26.56
13-S	Nitzschia	142.94	270.07	107.17	39.27	40.48
15-L	Nitzschia	17.39	101.65	18.65	11.09	20.88
15-S	Nitzschia	95.24	203.58	79.38	18.67	39.71
14-S	Carteria	126.21	220.71	84.34	26.68	36.73
16-L	Carteria	57.23	154.67	34.45	20.69	23.93
16-S	Carteria	57.16	204.68	74.75	34.75	34.42

TABLE 27 (Continued)

Eyes	Heart	Liver	Spleen	Alimentary Tract	Viscera	Ingested Food
168.36	86.15	75.28	14.29	56.88	100.27	64.60
367.78	14.29	101.21	72.22	134.20	75.74	16.42
146.34	41.98	67.49	--	67.05	104.49	97.38
299.06	22.22	64.62	18.12	86.25	184.82	29.41
251.51	38.96	102.76	--	81.67	54.13	78.38
198.12	23.44	96.37	38.57	75.45	117.59	72.13
285.34	--	47.87	--	69.86	296.32	66.67

TABLE 28

DECAY FISH--DISTRIBUTION OF TISSUE ACTIVITY* RANKED FROM HIGHEST TO LOWEST

Fish #	Algae Fed	Scales	Skin	Bone	Flesh	Gills	Eyes	Heart	Liver	Spleen	Alimentary Tract	Fiscera	Ingested Food
13-L	Nitzschia	10	2	9	12	8	1	4	5	11	7	3	6
13-S	Nitzschia	3	2	5	10	9	1	12	6	8	4	7	11
15-L	Nitzschia	10	3	9	11	8	1	7	5	12	6	2	4
15-S	Nitzschia	4	2	6	11	8	1	10	7	12	5	3	9
14-S	Carteria	3	2	5	11	10	1	9	4	12	6	8	7
16-L	Carteria	7	2	9	12	10	1	11	4	8	5	3	6
16-S	Carteria	7	3	4	9	10	2	11**	8	12**	5	1	6

*Units of activy = uc/gm dry weight.

**Equal ranking.

TABLE 29

DECAY FISH--DISTRIBUTION OF TISSUE ACTIVITY* RANKED FROM HIGHEST TO LOWEST EXCLUDING INGESTED FOOD

Fish #	Algae Fed	Scales	Skin	Bone	Flesh	Gills	Eyes	Heart	Liver	Spleen	Alimentary Tract	Viscera
13-L	Nitzschia	9	2	8	11	7	1	4	5	10	6	3
13-S	Nitzschia	3	2	5	10	9	1	11	6	8	4	7
15-L	Nitzschia	9	3	8	10	7	1	6	4	11	5	2
15-S	Nitzschia	4	2	6	10	8	1	9	7	11	5	3
14-S	Carteria	3	2	5	10	9	1	8	4	11	6	7
16-L	Carteria	6	2	8	11	9	1	10	4	7	5	3
16-S	Carteria	6	3	4	8	9	2	10**	7	11**	5	1

*Units of activity = uc/gm dry weight.

**Equal ranking.

TABLE 30

UPTAKE FISH--ANALYTICAL ERROR DATA

Analysis	Experimental Units (Aquaria) (Activity in units of 10^3 dpm/gm dry weight)							
	1		2		3		4	
	Fish 1	Fish 2	Fish 1	Fish 2	Fish 1	Fish 2	Fish 1	Fish 2
				Nitzschia				
1	32.8		21.8		16.3	17.0	18.6	
2	31.1		21.0		15.4	17.2	18.3	
Mean	31.95		21.40		15.85	17.10	18.45	
				Carteria				
1	49.2	21.0	23.6	40.0	50.0	30.2	39.9	37.1
2	49.8	21.2	23.9	40.6	49.6	30.4	39.8	37.2
Mean	49.50	21.10	23.75	40.30	49.80	30.30	39.85	37.15
				Control				
1	3.1	2.0	2.8	2.3	2.2	1.7	2.2	2.8
2	3.2	1.9	2.6	2.2	1.9	1.6	2.2	2.7
Mean	3.15	1.95	2.70	2.25	2.05	1.65	2.20	2.75

TABLE 31

UPTAKE FISH--COVARIANCE DATA

Fish No.	Aquaria							
	1		2		3		4	
	Activity	Length (cm)	Activity	Length (cm)	Activity	Length (cm)	Activity	Length (cm)
				Nitzschia				
1	31.95	10.0	21.40	9.1	15.85	10.5	18.45	10.0
2					17.10	10.0		
				Carteria				
1	49.50	8.0	23.75	9.5	49.80	8.5	39.85	9.7
2	21.10	10.9	40.30	8.8	30.30	9.7	37.15	10.4

Note: Activities expressed in units of 10^3 dpm/gm dry weight.

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This dissertation was prepared under the direction of the Chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Engineering and to the Graduate Council and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Date: December 18, 1965

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